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A FACTORIAL ANALYSIS OF THE PRENATAL GROWTH OF THE HUMAN SKELETON*

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(Received Oct. 20, 1958.)

GIVEN a normal environment, growth and development are under the rather rigid control of heredity. Viewing growth from the point of view of increase in the lengths of the shafts of the long bones, it is obvious that there are periods of acceleration and deceleration which characterize the ontogenetic pattern of each bone. Although differential rates of growth are peculiar to each bone, the timing and degree of acceleration or deceleration are not haphazard phenomena.

The characteristically human inter-relationships among the different bones of the adult skeleton are quite different from those of the newborn; and the latter, in turn, are different from those of the 3-month old fetus. The changing proportions of one bone to another that characterize the growth process and which ultimately lead to the specifically human adult proportions, are ultimately under the control of the genetic constitution. This is the only interpretation that seems reasonable in the light of investigations which show that fetuses of the same age group have proportional inter-relationships which significantly characterize that group (Kraus, 1958).

The specific question we pose in this paper is the following: Is the growth of the fetal skeleton under the control of a single growth regulatory field, or are there additional fields involved? Put another way, does a single "growth center" influence equally all areas of the fetal skeleton, or are there secondary fields whose influence is regionally specific?

Underlying the phenomenon of bone growth in general is what may be called a principal component or axis of bone growth. It is that axis or gradient which in mathematics is termed the least squares

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regression line. If one and only one axis is sought to describe most accurately the inter-relationships of the various bones of the fetal body as they occur through time, this line would be called the axis of the principal component. How accurately this axis describes the constellation of coordinates can be stated in terms of the percentage of variance of the coordinates that is accounted for by the principal component. Other axes may be explored to determine if they can account for the extra variance from the axis of the principal component.

For this study 118 human fetuses ranging from 30 to 169 mm. in crown-rump length were cleared and stained with alizirin red S. The lengths of the ossified shafts of the humerus, radius, femur, tibia, and the metacarpals and phalanges of the 3rd and 4th digits were measured by means of a micrometer disc inserted into a 15X eyepiece on a Cycloptic Stereoscopic Microscope. A maximum magnification of 80X could be obtained. Maximum morphological lengths were recorded. With the relatively low power of magnification used and with the "magni-changer" feature of the microscope which permits rapid "dial-in" selection of a wide range of magnifications, it was possible to observe the point of maximum extension of the calcified area at either end of a bone and to measure accurately to the nearest .05 mm. This degree of error did not affect the subsequent calculations. The fetuses ranged in age from 8 to 19 weeks (Patten, 1946, p. 184). Embryos smaller than 30 mm. were found to possess calcified shafts only for the bones of the arm and leg but not for the hands or feet.

The basic methodology (Hotelling, 1936) was as follows. Let $X_1 \ldots X_n$ be the variates and let r_{ij} be the element of correlation matrix. If we take any arbitrary set of numbers $A_1, A_2 \ldots A_n$ and form

$$A'_i = \sum_{j=1}^{n} r_{ij}A_j \qquad (i = 1, \ldots n) \qquad (1)$$

then A'_i is proportional to A_i if and only if they are proportional to one of the principal components (p_i) with the original variates.

If $A'_i = KA_i$ the K is the sum of the squares of the correlations of the X's with the particular principal component p_i. If $A'_i \neq KA_i$ then A'_i may be substituted into (1)

$$A''_m = \sum_{m=1} r_{mi}A'_i \qquad (i = 1, \ldots n) \qquad (2)$$

Repeat the same procedure. Then the ratios among the quantities obtained will eventually become and remain close to those among the coefficients of one of the a's. The component thus obtained in limit will be that having the greatest sum of squares of correlations with the X's. This is the coefficient of a_1 in the equation which gives the X's in terms of a's.

Defining the relationship

$$C_{mj} = \sum_i r_{mi} r_{ij}$$

and substituting (1) into (2) we get

$$A''_m = \sum_j C_{mj} A_j \qquad (3)$$

If R denotes the matrix of correlations, C_{mj} is the R^2. Hence substitution of a set of trial values in (1) is equivalent to multiplying it by the row of R, while substitution in (2) amounts to multiplication by the row of R^2. Similarly we can proceed by squaring R^2 and so forth to obtain K.

Putting C_1 for the matrix in which the element in the i^{th} row and j^{th} column is $A_{ij}A_{ji}$ (i, j = 1,2 . . . n), the matrix for finding the 2nd component R_1 is:

$$R_1 = R - C_1$$

In applying the foregoing argument to the data, we eliminated all specimens not having all 12 bones present. This left 118 fetuses. The 12 × 12 correlation matrix based upon 118 fetuses was obtained using Robinson's H-820 in the IBM 650 computer, and is presented in Table 1.

One can see how closely all 12 bones of the fetus are correlated with each other during the growth period from 8 to 19 weeks *in utero*.

Let R be the original 12 × 12 correlation matrix. To find the first

TABLE I
CORRELATION MATRIX

	Fem.	Tib.	Hum.	Rad.	Mc-3	Mc-4	PI-3	PI-4	PII-3	PII-4	PIII-3	PIII-4
Fem.	1.0000	.9920	.9875	.9889	.9744	.9736	.9702	.9698	.9239	.9131	.8783	.8874
Tib.	.9920	1.0000	.9881	.9899	.9713	.9699	.9661	.9656	.9218	.9123	.8801	.8908
Hum.	.9875	.9881	1.0000	.9855	.9661	.9664	.9587	.9588	.9165	.9057	.8657	.8765
Rad.	.9889	.9899	.9855	1.0000	.9723	.9712	.9654	.9651	.9249	.9133	.8745	.8865
Mc-3	.9744	.9713	.9661	.9723	1.0000	.9948	.9749	.9744	.9285	.9202	.8753	.8852
Mc-4	.9736	.9699	.9664	.9712	.9948	1.0000	.9741	.9733	.9223	.9144	.8722	.8806
PI-3	.9702	.9661	.9587	.9654	.9749	.9741	1.0000	.9965	.9276	.9166	.8705	.8790
PI-4	.9698	.9656	.9588	.9651	.9744	.9733	.9965	1.0000	.9290	.9184	.8669	.8763
PII-3	.9239	.9218	.9165	.9249	.9285	.9223	.9276	.9290	1.0000	.9779	.9328	.9349
PII-4	.9131	.9123	.9057	.9133	.9202	.9144	.9166	.9184	.9779	1.0000	.9256	.9256
PIII-3	.8783	.8801	.8657	.8745	.8753	.8722	.8705	.8669	.9328	.9256	1.0000	.9928
PIII-4	.8874	.8908	.8765	.8865	.8852	.8806	.8790	.8763	.9349	.9256	.9928	1.0000

principal component we compute R^2, R^4, and R^8. From R^8 we obtain the following sum of the row of the R^8 matrix; the trial value being 1:

(1)

268913265
268643022
266963160
268406889
268396725
267821795
267512084
267387288
263525355
261238586
253805035
255725150

We can divide each of these by the first, since this is the greatest:

(2)

1.00000
.99900
.99275
.99812
.99808
.99594
.99479
.99433
.97996
.97146
.94382
.95096

The products of these by the rows of R^8 are:

(3)

264957199
264690932
263935784
264457393
264448259
263388740
263576632
263453672
259648555
257395424
250071233
251963099

The product of this set by the row of R divided by the second of them are:

(4)

1.00000 (A_1)
.99900 (A_2)
.99275 etc.
.99811
.99808
.99596
.99479
.99433
.97996
.97146
.94382
.95096

These remain stationary under further iteration with R. Their products by the rows of R are:

$$
\begin{array}{lll}
11.29529 & (A'_1) = \sum_{j=1}^{12} r_{1j}A_j & \\
11.28393 & (A'_2) = \sum_{j=1}^{12} r_{2j}A_j & \\
 & \text{etc.} \qquad \text{etc.} & \\
11.21337 & & \\
11.27398 & & \\
11.27359 & & \\
11.24968 & & \\
11.23643 & & \\
11.23119 & & \\
11.06898 & & \\
11.09729 & & \\
10.66069 & & \\
10.74134 & &
\end{array} \qquad (5)
$$

The first value of the preceding, $K_1 = 11.29529$, corresponds to the value unity in the preceding set (4).

Multiplying each of the quantities (4) by the square root of the ratio of K_1 to the sum of the squares of these quantities,

$$\sqrt{\frac{11.29529}{11.64535}},$$

we obtain the correlations of the 12 bones with the first principal component or axis, p_1:

	Femur	.9849
	Tibia	.9839
	Humerus	.9777
	Radius	.9830
	Metacarpal 3	.9830
(6)	Metacarpal 4	.9809
	Phalanx I-3	.9797
	Phalanx I-4	.9793
	Phalanx II-3	.9651
	Phalanx II-4	.9568
	Phalanx III-3	.9295
	Phalanx III-4	.9366

The foregoing (6), then, are the coefficients of correlation of p_1 in the expressions for the bones in terms of the 12 principal components.

To compute the second principal component, multiply (6) by themselves and each other. We obtain:

$$C_1 = \begin{bmatrix}
.9700 & .9690 & .9629 & .9682 & .9682 & .9661 & .9649 & .9645 & .9505 & .9424 & .9154 & .9225 \\
.9690 & .9681 & .9620 & .9672 & .9672 & .9651 & .9639 & .9635 & .9496 & .9414 & .9145 & .9215 \\
.9629 & .9620 & .9559 & .9611 & .9611 & .9590 & .9579 & .9575 & .9436 & .9355 & .9088 & .9157 \\
.9682 & .9672 & .9611 & .9611 & .9663 & .9642 & .9630 & .9627 & .9487 & .9405 & .9137 & .9207 \\
.9682 & .9672 & .9611 & .9663 & .9663 & .9642 & .9630 & .9627 & .9487 & .9405 & .9137 & .9207 \\
.9661 & .9651 & .9590 & .9642 & .9642 & .9622 & .9610 & .9606 & .9467 & .9385 & .9117 & .9187 \\
.9649 & .9639 & .9579 & .9630 & .9630 & .9610 & .9598 & .9594 & .9455 & .9374 & .9106 & .9176 \\
.9645 & .9635 & .9575 & .9627 & .9627 & .9606 & .9594 & .9590 & .9451 & .9370 & .9103 & .9172 \\
.9505 & .9496 & .9436 & .9487 & .9487 & .9467 & .9455 & .9451 & .9314 & .9234 & .8971 & .9039 \\
.9424 & .9414 & .9355 & .9405 & .9405 & .9385 & .9374 & .9370 & .9234 & .9155 & .8893 & .8961 \\
.9154 & .9145 & .9088 & .9137 & .9137 & .9117 & .9106 & .9103 & .8971 & .8893 & .8640 & .8706 \\
.9225 & .9215 & .9157 & .9207 & .9207 & .9187 & .9716 & .9172 & .8961 & .8961 & .8706 & .8772
\end{bmatrix}$$

By the relation $R_1 = R\text{-}C$, we obtain R_1 for the computation of the 2nd principal component:

$$R_1 = \begin{bmatrix}
.0300 & .0230 & .0246 & .0207 & .0062 & .0075 & .0053 & .0063 & -.0320 & -.0283 & -.0411 & -.0351 \\
.0230 & .0319 & .0261 & .0227 & .0041 & .0048 & .0022 & .0021 & -.0278 & -.0291 & -.0344 & -.0307 \\
.0246 & .0261 & .0441 & .0244 & .0050 & .0074 & .0008 & .0013 & -.0271 & -.0290 & -.0431 & -.0392 \\
.0207 & .0227 & .0244 & .0389 & .0050 & .0074 & .0024 & .0024 & -.0238 & -.0272 & -.0392 & -.0342 \\
.0062 & .0041 & .0050 & .0050 & .0337 & .0306 & .0119 & .0117 & -.0202 & -.0203 & -.0384 & -.0355 \\
.0075 & .0048 & .0074 & .0070 & .0306 & .0378 & .0131 & .0127 & -.0244 & -.0241 & -.0395 & -.0381 \\
.0053 & .0022 & .0008 & .0024 & .0119 & .0131 & .0402 & .0371 & -.0179 & -.0208 & -.0401 & -.0386 \\
.0063 & .0021 & .0013 & .0024 & .0117 & .0127 & .0371 & .0410 & -.0161 & -.0186 & -.0434 & -.0409 \\
-.0320 & -.0278 & -.0271 & -.0238 & -.0202 & -.0244 & -.0179 & -.0161 & .0686 & .0545 & .0357 & .0310 \\
-.0283 & -.0291 & -.0298 & -.0272 & -.0203 & -.0241 & -.0208 & -.0186 & .0545 & .0845 & .0363 & .0295 \\
-.0411 & -.0344 & -.0431 & -.0392 & -.0384 & -.0395 & -.0401 & -.0434 & .0357 & .0363 & .1360 & .1214 \\
-.0351 & -.0307 & -.0392 & -.0342 & -.0355 & -.0381 & -.0381 & -.0409 & .0310 & .0295 & .1214 & .1228
\end{bmatrix}$$

Starting with trial values -1,-1,-1,-1,-1,-1,-1,-1, 1, 1, 2, 2, after we get R_1^4 using the same procedure as in computing the first principal component, we obtain the following stationary values:

(7)

— .373
— .336
— .395
— .354
— .324
— .350
— .338
— .347
.485
.513
1.000
.928

The products of these by the rows of R_1 are:

(8)

—.148239
—.133378
—.156794
—.140861
—.128605
—.140726
—.134015
—.138087
.192725
.203586
.397113
.368456

From (8) we find that $K_{11} = .397113$. Multiplying each of the values in (7) by the square root of the ratio of K_{11} to the sum of the squares of (7), $\sqrt{\frac{.39711}{3.3551}}$, we obtain the correlations of the 12 bones with the second principal component p_2:

Femur	—.128
Tibia	—.116
Humerus	—.136
Radius	—.122
Metacarpal — 3	—.111
Metacarpal — 4	—.120
Phalanx I-3	—.116
Phalanx I-4	—.119
Phalanx II-3	.167
Phalanx II-4	.177
Phalanx III-3	.344
Phalanx III-4	.319

By exactly similar methods, as in the first and second principal

components, we obtain the third and fourth components. The final results:

	p_1	p_2	p_3	p_4
Femur	.985	—.128	—.065	—.050
Tibia	.984	—.116	—.081	—.068
Humerus	.978	—.136	—.075	—.099
Radius	.983	—.122	—.064	—.081
Metacarpal — 3	.983	—.111	.023	.068
Metacarpal — 4	.981	—.120	.013	.075
Phalanx I-3	.980	—.116	.061	.117
Phalanx I-4	.979	—.119	.072	.108
Phalanx II-3	.965	.167	.156	—.067
Phalanx II-4	.957	.177	.186	—.094
Phalanx III-3	.930	.344	—.108	.050
Phalanx III-4	.937	.319	—.121	.046

From the above table the total percentage of the sum of the variance of each of the four principal components can be computed as

$$\frac{\sum_{i=1}^{12} p_{ij}^2}{12}$$

where i indicates *ith* bone and j indicates principal components. The percentage of the sum of the variance of the first principal component is then computed as

$$\frac{\sum_{i=1}^{12} p_{i1}^2}{12} = 94.1\%$$

and similarly we compute p_2, p_3, and p_4 to be 3.3%, 1.0%, and 0.6% respectively. The total sum is 99.0%. Since the first four principal components account for 99% of the total variance it was felt that the additional work involved in computing the remaining 8 components could be omitted.

The first principal component or axis accounts for 94.1% of the sum of the variances of the 12 bones. In particular the femur, humerus, radius, and tibia are most highly correlated with this component. Proceeding distally in the arm there is a decreasing gradient of correlation with the first component. It seems reasonable to regard this component as a general growth factor which is specifically human and whose dynamics are governed by the genetic constitution. In

other words it can be suggested that there is a major factor responsible for the characteristically human inter-relationships of the long bones at any point in time. The temporal changes that take place in these inter-relationships are the results of differential growth rates which are neither haphazard nor characteristic of any other species. The simplest explanation of this phenomenon is to be sought in the genetic system.

The percentages of the variance contributed by each component for each of the 12 bones have been computed. They are:

	Proportion of Variance				
	1st	2nd	3rd	4th	Sum
Femur	.970	.016	.004	.002	.993
Tibia	.968	.013	.007	.005	.993
Humerus	.956	.018	.006	.010	.990
Radius	.966	.015	.004	.007	.992
Metacarpal — 3	.966	.012	.0005	.005	.984
Metacarpal — 4	.962	.014	.0002	.006	.982
Phalanx I-3	.960	.013	.004	.014	.991
Phalanx I-4	.958	.014	.005	.012	.989
Phalanx II-3	.931	.028	.024	.004	.988
Phalanx II-4	.916	.031	.035	.009	.991
Phalanx III-3	.865	.118	.012	.002	.997
Phalanx III-4	.878	.102	.015	.002	.996

It is interesting to note that those bones are least correlated with the principal component which are most distally located on the arm. There is a gradient in this respect also from proximal to distal.

The second principal component is concerned primarily with the most distal segments, phalanges III-3 and 4, and accounts for 12% and 10% respectively of their variance. A slight proportion of the variance of the middle phalanges is also described by the second principal component.

The third principal component is a factor primarily in the growth characteristics of the middle phalanges, while the fourth component concerns the proximal phalanges. Apparently the fifth principal component would indicate an axis descriptive of metacarpal growth.

With only four principal components it is obvious that most of the variance of the 12 bones is accounted for. In the case of the major long bones, from 96-97% of the variance is due to the first component, the general growth axis. Both the first and second components account for 98% of the variance of the distal phalanges. The first, sec-

ond, and third components account for 98% of the variance of the middle phalanges. In the case of the proximal phalanges, the first, second, and fourth components account for 98% of the variance. All four components are required to account for 98% of the variance of the metacarpals. The sums of the percentages of the four components for each bone indicates that only a negligible proportion of the variance remains unaccounted for and this would be distributed in very small amounts over the remaining 8 components.

The most logical explanation of these phenomena appears to rest upon a genetic interpretation. The changing metrical inter-relationships of the bones of the fetal skeleton during growth are regulated primarily by a general growth mechanism or field of influence under genetic control. The influence of this mechanism is exerted early in the process of bone formation and diminishes slightly toward the periphery of the skeleton. Over and above this major field of influence certain areas of the skeleton come under regional fields of influence, also under genetic control. Thus there is clear indication that the phalanges and metacarpal of a single digit are not under the same field of influence other than with respect to the general growth factor. Instead, the distal phalanges are secondarily influenced by one set of factors, the middle phalanges by another, and so on.

If our interpretation of these growth factors as separate genetic regulators or controls is correct, then we should be able to observe their phenotypic effects when a mutant occurs in the system. In other words, if one of the postulated growth factors, say the second component, which exerts a distinguishable but secondary effect on the distal phalanges, is affected by a mutant gene, we might expect to observe abnormal growth characteristics in the distal phalanges alone. The same would apply to the third component whereby we would look for irregularities in the growth of the middle phalanges, etc.

It is interesting and suggestive that numerous hereditary abnormalities are known which meet these specifications. Apical dystrophy, for example, is a simple Mendelian dominant character characterized, among other things, by a reduction in size of the distal phalanges. It may act apico-basally in direction. Other forms of brachydactyly occur in which only the metacarpals (brachymetapody), or only the middle phalanges (brachymesophalangy), are reduced in size or absent (MacArthur and McCullough, 1932). A thorough review of the many types and pedigrees of brachydactyly and an attempt at an etiological

interpretation have been made by Pol (1921). There seems to be general agreement that the involved gene acts as an inhibitor of growth, the degree and extent of reduction being related to the critical point in time.

It is tempting to read into the data presented herein a correlation between the various growth components and the clinical evidence of regional growth influence of individual genes. That the various long bones of the human fetal skeleton are under a major regulatory control—what we have called the principal component—seems quite evident. In addition, however, it has been demonstrated that there are regional characteristics of growth which are under the influence of secondary factors or components. That the genetic constitution in some way is to be identified with these components seems highly probable.

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VASCULOGENESIS IN SELECTED ENDOCRINE GLANDS OF NORMAL AND HYPOPHYSECTOMIZED CHICK EMBRYOS. I. THE THYROID[1]

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INTRODUCTION

THE internal vascularity of endocrine glands is an exceedingly important constituent of the endocrine system. It is the immediate source of raw materials for synthesis of hormones, and in many instances it provides trophic hormones which stimulate development and synthetic activities of glandular cells. Moreover, the hormones are transferred to the blood stream, thereby initiating delivery to their sites of action.

Willier (1955) recently discussed the possibility that changes in the vascular pattern of developing endocrine glands might serve as an index of time of onset of their functional activity. He reviewed some of the evidence in support of this hypothesis. Increases in vascularity immediately prior to onset of function were noted in developing thyroids of mammals (Rankin, 1941, in pig embryos; Gorbman and Evans, 1943, in rats; Koneff *et al*, 1949, in bovine fetuses). The vascular pattern of the adrenal cortex of pigs and dogs changes from an irregular plexus of capillaries in early stages of development to a longitudinally oriented pattern of vessels in later stages (Flint, 1900; Whitehead, 1933). Similar changes in vascular patterns in adrenals of rats and mice occur at a time when the cortical cells are first assuming their definitive arrangement, thereby suggesting a correlation between time of establishment of function and an orientation of blood vessels which is conducive to a more efficient flow of

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blood (Gersh and Grollman, 1941). The islets of chromaffin cells are provided with a special capillary network of their own as they begin to migrate through the cortex. As the islets increase in size and move inward the capillary network of each of them shifts its connections in such a way that a more efficient flow of blood is provided (Flint, 1900; Willier, 1955). In the albino rat (Hard, 1944) the beta cells of the pancreas, generally accepted as the source of insulin in adult animals, are first identified in 18½-day fetuses within a few hours after a capillary network has developed within the larger islets. Beta granules soon accumulate on the side of the cell towards the capillary (Willier, 1955).

A considerable amount of information has accumulated concerning histogenesis in thyroid glands of chick embryos and certain morphological signs of thyroid activity have been described (production of colloid, appearance of chromophobic vacuoles in the colloid, increased height of epithelial cells at certain stages, etc.). More recent studies on chick embryos conducted with the aid of radioactive iodine (I^{131}) have added much information about the developmental stages at which this element is first accumulated selectively and is first bound organically by the embryonic thyroid. They have also indicated the stages in development when monoiodotyrosine, diiodotyrosine, and thyroxine can first be detected in embryonic thyroids. It is the purpose of the present study to describe vasculogenesis in thyroid glands of normal chick embryos and to compare the changes in vascularity with known changes in histogenesis and functional activity of these glands. In this way it should be possible to determine to what extent significant correlations do occur between changes in vascularity and changes in functional activity of an endocrine gland.

The present paper will also examine development of the vascular pattern in thyroid glands of hypophysectomized chick embryos. Development of the thyroid gland is modified following hypophysectomy (Fugo, 1940; Martindale, 1941): the gland never enlarges beyond the size attained by normal 14-day embryos; follicles are relatively few in number, small in size, and contain little or no colloid. Similarly chick thyroids grown *in vitro* under conditions which eliminate the possibility of providing thyrotrophic hormone either via blood plasma or embryonic extract (Tixier-Vidal, 1955) are morphologically and probably biochemically deficient as indicated by reduced size and number of follicles and reduced amount of colloid. Such de-

ficiencies are corrected if pituitary glands are grown adjacent to the thyroid *in vitro* (Tixier-Vidal, 1955, 1956). If changes in vascular pattern are correlated with changes in functional activity of embryonic thyroid glands, development of the vascular pattern would be expected to differ in hypophysectomized and in control embryos.

Materials and Methods

All embryos were obtained from White Leghorn eggs. Incubation occurred at a temperature of 101 ±2 degrees F. in forced draft incubators. The ages of all specimens represent the actual length of time the eggs remained in the incubators. Hypophysectomy was accomplished at 33 to 38 hours of incubation by the partial decapitation method of Fugo (1940).

All specimens were injected with undiluted India ink with the aid of small capillary pipettes; pressure was applied to these pipettes by soft rubber bulbs. The shell was carefully removed from an area large enough to allow access to the larger extraembryonic veins. The vein selected was grasped with a watchmaker's forceps and the capillary tube was inserted into the vein just proximal to the point of grasping. The vein was then released from the forceps and the ink introduced slowly by gentle pressure applied to the rubber bulb. The capillary tube was then withdrawn and the egg left undisturbed for a few minutes to permit the ink to circulate uniformly to all parts of the body. Immediately thereafter the embryos were gently removed from the eggs, rinsed in saline, and fixed in Bouin's solution. Control specimens were preserved at daily intervals, from the tenth through the eighteenth days of incubation; hypophysectomized embryos were preserved at daily intervals from the tenth through the sixteenth days, and also on the eighteenth day. Twenty-eight hypophysectomized embryos were injected successfully.

Thyroid glands were carefully dissected from preserved embryos. Such isolated tissues were hydrated and stained *in toto* with borax carmine. Subsequently they were dehydrated, cleared, and embedded in nitrocellulose. All specimens were sectioned at 60μ and were mounted serially according to the method of Feeney (1944). In addition to the specimens prepared specifically for the present study there was available the series of normal injected chick embryos prepared by Feeney and Watterson (1946). These had been incubated 2½, 3, 3½, 4, 5, 6, 7, 9, 10, 12, 14 and 16 days. The younger specimens were

especially valuable since they enabled the author to examine the initial steps involved in the elaboration of the characteristic vascular pattern.

All photomicrographs were taken with Kodachrome film; negatives were made from the transparencies and printed upon photographic paper designed for printing low contrast negatives. These steps were taken in order to increase contrast in the final prints.

Observations

Control embryos. The thyroid gland of the chick embryo arises as a small, spherical evagination of the epithelium of the floor of the pharynx situated between, and a little in front of, the ventral ends of the second pair of visceral pouches (Hamilton, 1952). Evagination begins at about the 26-somite stage (approximately 51 hours). The connections between the thyroid gland and the pharynx gradually close until on the fourth day the thyroid sac loses all continuity with the pharyngeal epithelium, whereupon it forms a small spherical vesicle lying beneath the floor of the pharynx.

In the two 4-day specimens of the Feeney-Watterson series this vesicle has subdivided completely into right and left lobes, somewhat earlier than described by Hamilton (1952), Schrier and Hamilton (1952), and others. Each of the paired masses of thyroid tissue appears quite solid and homogeneous. In one specimen no capillary plexus has formed on the outer surface of the lobes; in the other a vascular plexus is clearly forming on the outer surface of each lobe. In the 5-day specimens each lobe of the thyroid gland has moved dorsally toward the trachea and a vascular plexus is present on the outer surface of each; no blood vessels have penetrated into the substance of the gland as yet. The two masses of thyroid tissue in 6-day embryos have enlarged considerably. Numerous vessels have penetrated into each lobe of the thyroid and have anastomosed with one another (Fig. 1). Even though the lobes still have a rather homogeneous appearance, they are not uniformly supplied with vessels at this stage.

After 7 days of incubation the networks of blood vessels are more uniformly distributed throughout the lobes (Fig. 2) and the individual vessels appear to be increasing in diameter. This network of blood vessels separates the thyroid substance into plates of cells.

These strands of thyroid tissue are even more sharply delineated in 8-day specimens (Fig. 3). By this time the vascular pattern within each lobe is clearly sinusoidal in character for the diameters of these vessels have increased markedly, but not uniformly, along their lengths. Thyroids of embryos incubated 9 days are characterized by somewhat narrower strands of thyroid cells which become increasingly isolated from one another by the ever-increasing diameters of the sinusoids (Fig. 4). In 10-day embryos these cellular cords have subdivided into small clumps of thyroid cells (Fig. 5), and the gland consists primarily of large sinusoids separating small islands of glandular tissue. Thyroids of 11-day specimens reveal a decided increase in the amount of thyroid tissue (Fig. 6) and a simultaneous decrease in the diameter of blood vessels, although these vessels are still clearly sinusoidal in nature (compare with Fig. 208, p. 581, in Willier, 1955).

After 11 days of incubation thyroid follicles become increasingly prominent. At the same time the vascular channels decrease in diameter and as a consequence there is established a vascular pattern which is increasingly capillary in nature (Fig. 7). From the fifteenth through the eighteenth days of incubation there occurs a progressive increase in the number and size of follicles. Meanwhile almost all traces of the earlier sinusoidal circulation disappear; the definitive vascular pattern now emerges clearly with each follicle sharply bounded by a capillary network (Figs. 11 and 13; compare with Fig. 16-6, p. 307 in Maximow and Bloom, 1957).

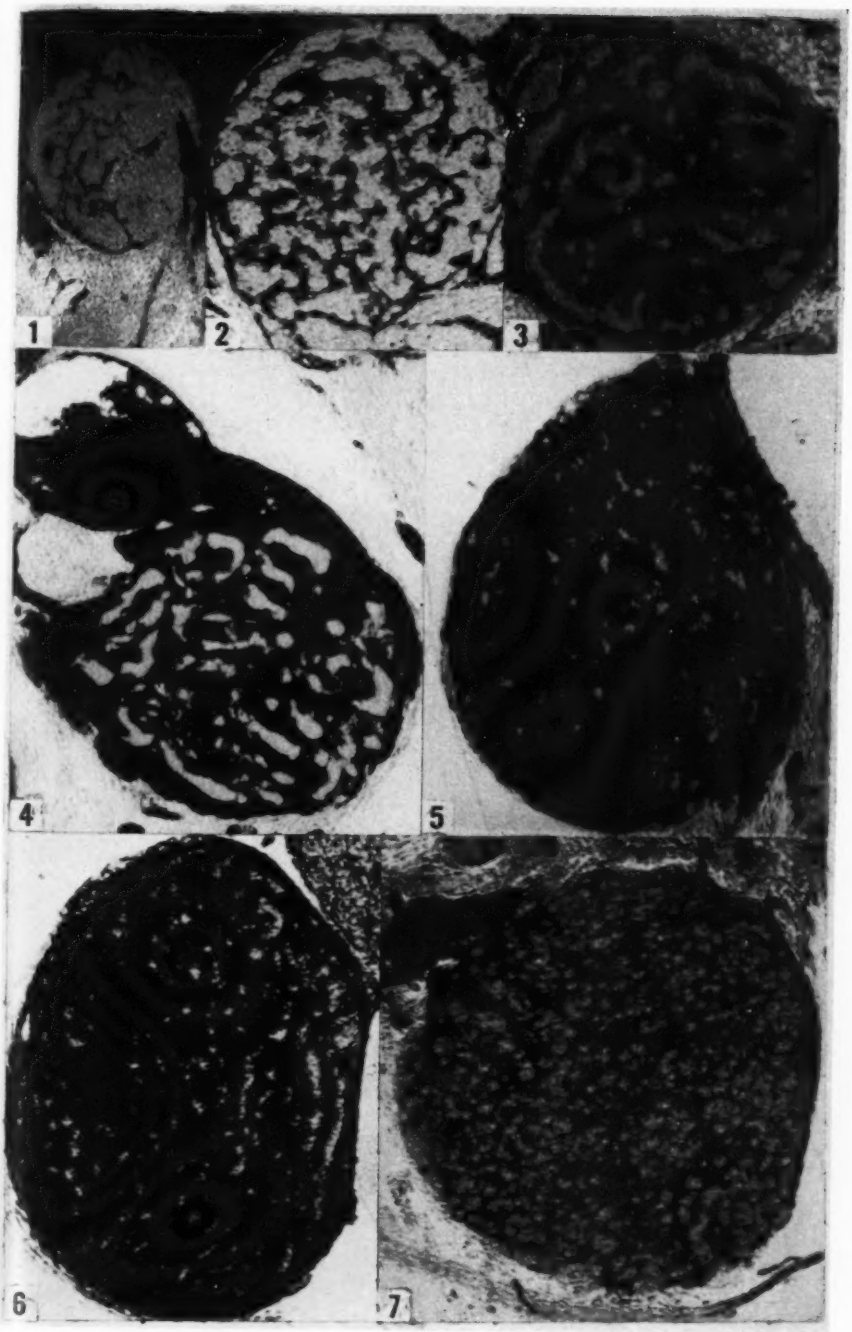
Thus a careful study of the development of the vascular pattern within each lobe of the thyroid gland reveals the rapid formation of a distinctive sinusoidal network from the sixth to the eighth days of incubation, maximal development of this sinusoidal circulation in 9- and 10-day embryos, and a progressive conversion of this sinusoidal network into a network of capillaries beginning on the eleventh or twelfth days of incubation. The definitive vascular pattern of the thyroid gland appears to be established by 16 to 18 days of incubation.

Hypophysectomized embryos. Early stages in development of the vascular pattern in thyroid glands of hypophysectomized embryos are essentially identical with those described in normal embryos (compare Fig. 8 with Fig. 5). By 11 days of incubation vasculogenesis in thyroids of hypophysectomized embryos (Fig. 9) begins to lag behind that of control embryos of the same age (Fig. 6). The prominent sinusoids still present in thyroid glands of hypophysectomized em-

PLATE 1

Explanation of Figures

1. Specimen *CI 6-2-11*. Cross section of the thyroid of a normal 6-day embryo. Enlargement 100.8X.
2. Specimen *CI 7-2-22*. Cross section of the thyroid of a normal 7-day embryo. Enlargement 144X.
3. Specimen *CI 8-1-31*. Cross section of the thyroid of a normal 8-day embryo. Enlargement 144X.
4. Specimen *CI 9-2-41*. Cross section of the thyroid of a normal 9-day embryo. Enlargement 97.2X.
5. Specimen *CI 10-5*. Cross section of the thyroid of a normal 10-day embryo.
6. Specimen *CI 11-6*. Cross section of the thyroid of a normal 11-day embryo. Enlargement 144X.
7. Specimen *CI 14-2-1*. Cross section of the thyroid of a normal 14-day embryo. Enlargement 108X.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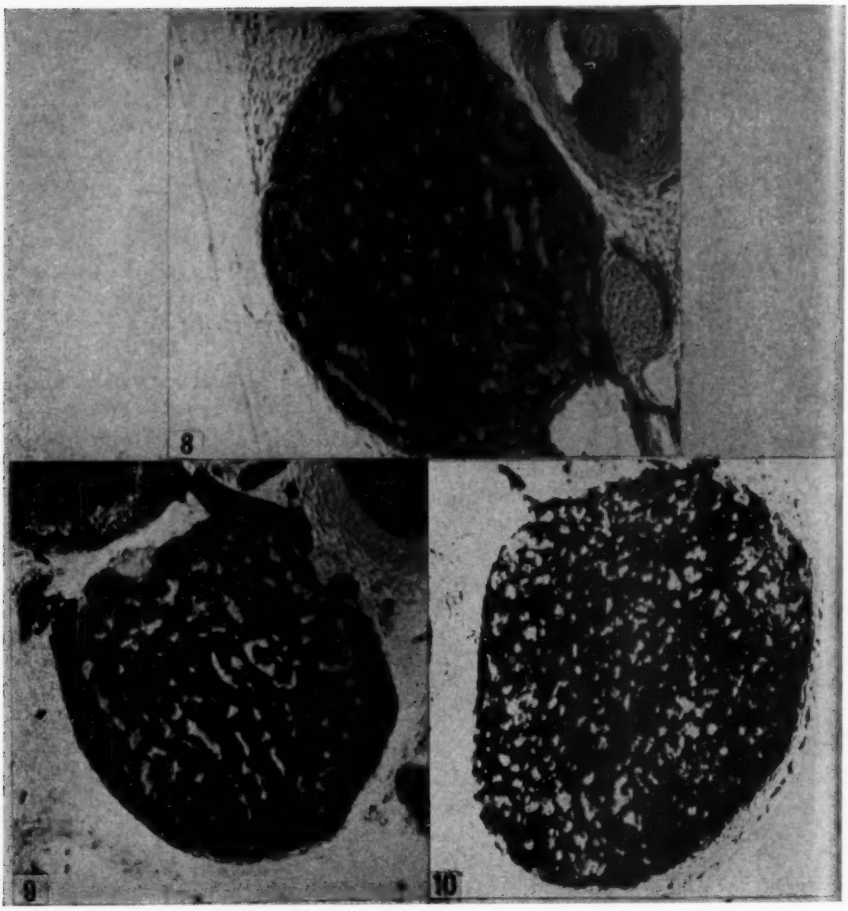

PLATE 2

EXPLANATION OF FIGURES

8. Specimen *10-4*. Cross section of the thyroid of a 10-day hypophysectomized embryo. Enlargement 144X.
9. Specimen *11-3*. Cross section of the thyroid of an 11-day hypophysectomized embryo. Enlargement 144X.
10. Specimen *12-6-1*. Cross section of the thyroid of a 12-day hypophysectomized embryo. Enlargement 97.2X.

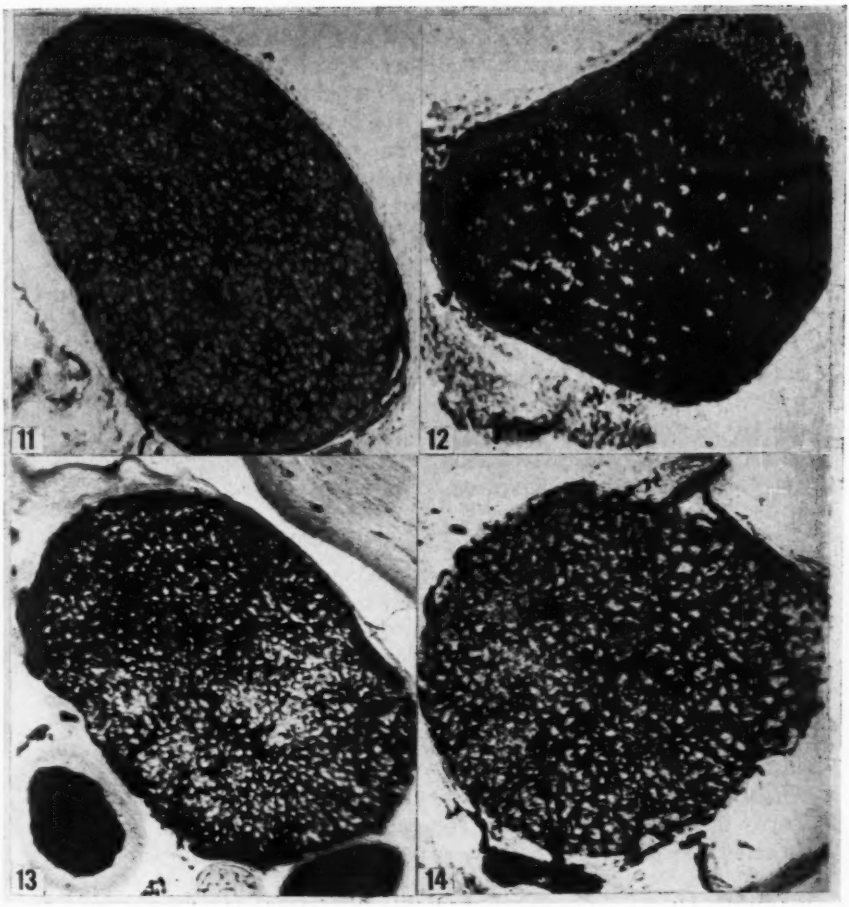

PLATE 3

EXPLANATION OF FIGURES

11. Specimen *CI 16-6-1*. Cross section of the thyroid of a normal 16-day embryo. Enlargement 79.2X.
12. Specimen *15-2*. Cross section of the thyroid of a 15-day hypophysectomized embryo. Enlargement 96X.
13. Specimen *CI 18-2-4*. Cross section of the thyroid of a normal 18-day embryo. Enlargement 72X.
14. Specimen *18-4*. Cross section of the thyroid of a 18-day hypophysectomized embryo. Enlargement 79.8X.

bryos at this time are almost identical in appearance with those of 9- and 10-day control embryos (Figs. 4 and 5). The vascular pattern of 12-day hypophysectomized embryos (Fig. 10) has advanced little, if at all, beyond that characteristic of normal 11-day embryos (Fig. 6), and the vascular pattern of 15-day hypophysectomized embryos (Fig. 12) differs in no way from that of normal 11-day embryos. Associated with retention of the sinusoidal vascular pattern at this advanced stage is the almost complete failure of follicle formation in thyroid glands of hypophysectomized embryos. This is in striking contrast with the situation in 14-day control embryos (Fig. 7) where formation of follicles containing colloid is clearly under way and where transformation of the sinusoidal vascular pattern into a network of capillaries is becoming apparent. Even after 18 days of incubation the vascular pattern of thyroid glands of hypophysectomized embryos (Fig. 14) remains clearly sinusoidal in nature, and most of the clusters of cells have failed to form follicles. Consequently there is a marked contrast between the vascular patterns of thyroid glands of 18-day hypophysectomized embryos (Fig. 14) and of 18-day control embryos (Fig. 13).

Thus the most striking difference between vascular patterns of thyroid glands of hypophysectomized and control embryos is the complete failure of the sinusoidal circulation of the former to transform into the capillary circulation of the latter after 14 or 15 days of incubation.

Relation of Vasculogenesis to Histogenesis

Control embryos. The literature contains many observations on histogenesis in thyroid glands of normal chick embryos. These observations on vasculogenesis of thyroid glands of normal chick embryos have been recorded in the past, but no systematic study of injected specimens has been reported. All available observations on vasculogenesis, both those of the present study and those available in the literature, are likewise summarized in Table 1. The former are printed in capital letters.

It is evident from this tabular summary of the relation of vasculogenesis to histogenesis in normal embryos that changes in the vascular pattern are intimately associated with changes in development of the thyroid epithelium. The initial penetration of blood vessels into the thyroid gland may be a factor in subdividing the solid mass of thyroid

TABLE I

HISTOGENESIS, VASCULOGENESIS, AND FUNCTIONAL DIFFERENTIATION IN THYROID GLANDS OF NORMAL EMBRYOS

Incubation age in days	Histogenesis	Vasculogenesis	Functional Differentiation
5	Gland divided into 2 solid lobes separated from each other and from pharynx (Bradway, 1929; Hopkins, 1935).	VASCULAR PLEXUS ON OUTER SURFACE OF EACH LOBE; NO PENETRATION OF VESSELS INTO SUBSTANCE OF GLAND.	Thyroid accumulates 50X more I^{131} than heart muscle; little or no organic binding of I^{131}; probably no conversion to iodine (Trunnell and Wade, 1955).
6	Thyroid cells grouped into cords (Sun, 1932; Venzke, 1949) or plates (Hopkins, 1935). Non-follicular spaces present (Bradway, 1929; Venzke, 1949) and become invaded by mesenchyme (Hopkins, 1935; Venzke, 1949); these may be blood vessels.	Circulatory spaces separate plates of epithelial cells (Hopkins, 1935). NUMEROUS VESSELS HAVE PENETRATED EACH LOBE AND HAVE ANASTOMOSED WITH ONE ANOTHER, BUT VASCULAR NETWORK IS NOT UNIFORM THROUGHOUT GLAND.	
7	Cellular cords of double thickness (Bradway, 1929; Venzke, 1949; Stoll, and Blanquet, 1952a, 1952b). Cords thickly packed, twisted, and convoluted (Kraicziczek, 1956a). A few PAS positive intracellular colloid droplets (Wollman and Zwilling, 1953).	Erythrocytes already found in narrow capillaries; beginning of circulation is established (Kraicziczek, 1956a). NETWORKS OF BLOOD VESSELS UNIFORMLY DISTRIBUTED THROUGHOUT GLAND; INDIVIDUAL VESSELS INCREASING IN DIAMETER.	Power of thyroid to concentrate I^{131} superior to that of cardiac muscle (Stoll and Blanquet, 1952a, 1952b); its content of I^{131} about 18X that of blood. Concentration of I^{131} averaged 400 to 700X that of blood, but no organic binding as yet (Wollman and Zwilling, 1953).
8	Thick convoluted cords of cells; very small amount of conn. tissue; no indication of colloid droplets or follicle formation (Carpenter, 1942). First morphological signs of secretion (Studitskii, 1946). PAS positive droplets present (Carpenter, *et al.*, 1954). Strandlike, twisted cell bands; first intracellular droplets (Kraicziczek, 1956a).	Blood spaces separate cords of cells (Carpenter, 1942). Active circulation of gland evident from numerous erythrocytes in capillaries; make transport of hormone away from gland possible and probable (Kraicziczek, 1956a). VASCULAR PATTERN WITHIN EACH LOBE CLEARLY SINUSOIDAL IN CHARACTER. DIAMETERS OF VESSELS HAVE INCREASED MARKEDLY BUT NOT UNIFORMLY ALONG THEIR LENGTHS.	Thyroid responds to thyrotrophic action of chorioallantoic grafts of pituitary glands (Studitskii, 1946). Some capacity to concentrate I^{131}; little if any organic binding (Carpenter *et al.*, 1954); 7-day thyroids cultured one day form monoiodotyrosine (Gonzales, 1954); monoiodotyrosine first synthesized at 8½ days (Trunnell and Wade, 1955).

TABLE 1 (*continued*)

Incubation age in days	Histogenesis	Vasculogenesis	Functional Differentiation
9	Vesicles appear; epithelial cells of cuboidal type (Sun, 1932). Number of cords with double rows of nuclei increases; no colloid apparent (Carpenter, 1942). Some cells contain droplets staining blue in azan (Wingstrand, 1951). PAS preparations show many deep magenta droplets whose diameter is *ca* half that of nucleus (Wollman and Zwilling, 1953). Intracellular colloid demonstrated from now on with MacManus stain (Tixier-Vidal, 1955).	Blood spaces becoming larger giving the section a fenestrated appearance (Carpenter, 1942). Practically all nonfollicular spaces contain blood cells; spaces enlarged in size (Venzke, 1949). STRANDS OF CELLS INCREASINGLY ISOLATED FROM ONE ANOTHER BY EVER INCREASING DIAMETERS OF SINUSOIDS.	At least half administered I^{131} protein-bound after 80 minutes and at least 90% after 3½ hours; concentration of I^{131} averaged over 5000X that of blood (Wollman and Zwilling, 1953). 7-day thyroids cultured 2 days synthesize diiodotyrosine and thyroxine (Gonzales, 1954) in addition to monoiodotyrosine. Diiodotyrosine first appears at 9¼ days and thyroxine at 9¾ days (Trunnell and Wade, 1955).
10	First colloid chromophilic; in form of droplets of smaller size than nuclei; accumulates between 3 or 4 cells whose boundaries are still indistinct (Hopkins, 1935). Cords loosely convoluted, mostly 2 cells wide; first appearance of clearly distinguishable droplets of colloid within cells (Carpenter, 1942). Glandular tissue has syncytial appearance; rearrangement of cells to form spherules; occasional primary follicle; increase in number of cells showing chromophobic vacuoles (Venzke, 1949). Colloid first appears as tiny droplets (Martindale, 1941). Nests of epithelial cells surrounded by vascular conn. tissue; no discrete follicles (Hansborough and Khan, 1951). Follicles begin to form (Bartels, 1944). Intracellular droplets of colloid and first follicles (Stoll and Blanquet, 1952a). Colloid in rounded and homogeneous droplets,	Follicles separated from one another by large blood sinuses (Bartels, 1944). Organ still richer in capillaries than on 9th day (Kraicziczek, 1956a). CELLULAR CORDS HAVE SUBDIVDED INTO SMALL CLUMPS OF THYROID CELLS AND GLAND CONSISTS OF LARGE SINUSOIDS SEPARATING SMALL ISLANDS OF GLANDULAR TISSUE. MAXIMAL DEVELOPMENT OF SINUSOIDAL CIRCULATION ON 9th AND 10th DAYS.	Thyroid of 10-day embryo produces metamorphosis when fed to tadpoles (Hopkins, 1935). Anterior pituitary grafted to chorioallantoic membrane clearly stimulates thyroid (Studitskii, 1946). 8-day thyroids cultured 2 days *in vitro* accumulate I^{131} vigorously (Gaillard, 1953). In athyroid embroys thyroxine in rest of body (Meyniel, Blanquet, Mournier, Stoll, and Maraud, 1955). Thyrotrophic hormone level of blood attains threshold of sensitivity of early thyroid (Tixier-Vidal, 1956). Thyroxine present in 7- to 7½-day thyroids cultured 3 days (Gonzales, 1956); none of iodinated compounds in either protein or nonprotein fraction of medium. Thyroxine present in easily detectable amounts (Trunnell and Wade, 1955). I^{131} uptake curve increases slope between 10th and 11th days (Trunnell and Brayer, 1953). Ac-

TABLE 1 (*continued*)

Incubation age in days	Histogenesis	Vasculogenesis	Functional Differentiation
10 (cont.)	sometimes intracellular, often situated at limit between 2 or 3 cells (Tixier-Vidal. 1955). Beginning of follicle formation; colorless chromophobic vacuoles appear in homogeneous colloid (Kraicziczek, 1956a).		cumulation of I^{131} increases suddenly and considerably between 10-13 days (Stoll and Blanquet, 1952a). Thyroid cells synthesize thyroxine between 10th and 12th days (Blanquet, Stoll, Maraud, and Capot, 1953a, 1953b).
11	Primary follicles and prefollicular globules seen for first time; follicle cells typically cuboidal, nuclei peripherally located (Bradway, 1929). Follicular cavities formed by secretion and coalescence of globules of chromophilic colloid which originates on side of nucleus next to prospective lumen; chromophobic colloid appears; increased breaking up of tissue which later form follicles, and rearrangement of cells results in formation of irregular columns (Hopkins, 1935). Primitive follicles appearing as groups of cells within a cord and are not cut off at this time (Carpenter, 1942). Discrete follicles (Hansborough and Khan, 1951). Increased height of follicle cells, increase in vacuoles in colloid (Bartels, 1944). Chromophobic vesicles still more evident; almost 100% of follicles show them (Kraicziczek, 1956a).	Thyroid tissue becoming compact; nonfollicular spaces decreased. This may be caused by increase in glandular cells following excess of mitotic activity (Venzke, 1949). Whole organ has still more capillaries than on 10th day and has a very rich flow of blood (Kraicziczek, 1956a). DECIDED INCREASE IN AMOUNT OF THYROID TISSUE AND SIMULTANEOUS DECREASE IN DIAMETER OF BLOOD VESSELS, ALTHOUGH LATTER ARE STILL DISTINCTLY SINUSOIDAL IN CHARACTER. ON 11th AND 12th DAYS A PROGRESSIVE CONVERSION OF SINUSOIDAL NETWORK INTO NETWORK OF CAPILLARIES BEGINS.	Thyrotrophic hormone produced in effective amounts at least as early as the 11th day; self-differentiation of thyroid prior to this stage is indicated (Martindale, 1941). Autoradiograms of whole thyroid show intense activity (Hansborough and Khan, 1951). Strong radioautographs from 8½-day thyroids cultured 3 days (Gaillard, 1953). Body growth retarded on 11th day when thiourea given at 7 days; may indicate deficiency of thyroid hormone in presence of thiourea (Vidal, 1952).
12	Colloid of gland mostly chromophilic; previously was chromophobic (Bradway, 1929). A few distinctly outlined follicles (Carpenter, 1942). Follicles typical of adult now present (Venzke,	AFTER 11 DAYS FOLLICLES BECOME INCREASINGLY PROMINENT. AT THE SAME TIME VASCULAR CHANNELS DECREASE IN DIAMETER AND AS A CONSE-	Small amount of thyroxine present, but appears to be more diiodotyrosine than thyroxine at this stage (Blanquet, Stoll, Maraud, and Capot, 1953a, 1953b). Melanotic condition develops in down

TABLE 1 (*continued*)

Incubation age in days	Histogenesis	Vasculogenesis	Functional Differentiation
12 (cont.)	1949). Maximal height of follicle cells (Bartels, 1944; Kraiczicczek, 1956a).	QUENCE THERE IS ESTABLISHED A VASCULAR PATTERN WHICH IS INCREASINGLY CAPILLARY IN NATURE.	feathers of hypophysectomized chick embryos; can be corrected by injection of thyrotrophic hormone (Fugo, 1940).
13	Follicles distinctly outlined for first time (Bradway, 1929). From 13th to 16th days more follicles become clearly delineated and all stage of follicle formation can be observed (Carpenter, 1942). Chains of follicles (Bartels, 1944).	Inside gland capsule is a large sinus continuous with circulatory spaces between convoluted epithelial plates; although they contain erythrocytes, spaces between plates more nearly approximate lymph spaces (Hopkins, 1935).	Reduced capacity to accumulate I^{131} in hypophysectomized chick embryos (Maroud, Stoll, and Blanquet, 1957).
14	Single follicles separate from their attachment, slide together, and press closely against each other so that gland acquires a very compact appearance; gland acquires its definitive structure (Bartels, 1944).	Most circular follicles press tightly against one another leaving only small blood vessels between. Only slit-like blood spaces remain between outer walls of follicles; not much larger than blood corpuscles (Bartels, 1944). By 14⅔ days vascular spaces surround part of every follicle (Venzke, 1949).	
15	Gland has attained essentially same appearance as at hatching (Martindale, 1941) and as in adult (Sun, 1932).	Lymph spaces between follicles (Bradway, 1929). Follicles all in contact with vascular spaces at 15⅞ days (Venzke, 1949). There is a progressive decrease in vascularity accompanying increase in the number of follicles (Ferguson *et al.*, 1957). FROM THE 15th THROUGH THE 18th DAYS ALL TRACES OF THE EARLIER SINUSOIDAL CIRCULATION DISAPPEAR. DEFINITIVE VASCULAR PATTERN NOW EMERGES CLEARLY WITH EACH FOLLICLE SHARPLY BOUNDED BY A CAPILLARY NETWORK.	Amount of thyroxine on radiochromatogram greater than amount of diiodotyrosine (Blanquet, Stoll, Maraud, and Capot, 1953a, 1953b).

TABLE 1 (*continued*)

Incubation age in days	Histogenesis	Vasculogenesis	Functional Differentiation
16	Distinctly outlined follicles throughout thyroid, although traces of cord-like structure can still be seen (Carpenter, 1942).	Intrafollicular capillaries have a rich flow of blood (Kraicziczek, 1956a).	Colloid reabsorption beginning on the 16th day leads to a typical turbulent outflow on 18th day (Kraicziczek, 1956a).
18	Maximal height of follicle cells at 17½ days (Bartels, 1944). Maximal height of follicle cells, presence of chromophobic vacuoles, appearance and position of nuclei, size and position of Golgi apparatus, all indicate precipitate activity of thyroid (Kraicziczek, 1956a).	At 18¾ days glandular tissue is very compact; has crowded many of the vascular spaces out of existence. Epithelium lining these spaces remains between follicles forming a basement membrane for follicle cells. Largest vascular spaces at periphery of gland. Many still contain erythrocytes (Venzke, 1949). Slit-like interfollicular spaces provide for rich blood supply and rapid transport (Kraicziczek, 1956a).	Values of both percentage and absolute iodine content seem to drop off (Sun, 1932). Sharp decrease of iodine content shows precipitous colloid outflow (Kraicziczek, 1956a). Slight fall in concentration of thyroxine in gland on 17th to 20th days (Trunnell and Wade, 1955). Thyroid output causes decline in growth and increase in differentiation; rapid keratinization of skin sign of thyroxine activity (Kraicziczek, 1956b). Thyroxine seems necessary for resorption of yolk, withdrawal of yolk sac, and initiation of hatching (Grossowicz, 1946; Adams and Bull, 1949; Vidal, 1952; Romanoff and Laufer, 1956).

epithelium into cords or plates of cells, or it may be that the rearrangement of the solid mass of thyroid epithelium into cords or plates is an intrinsic characteristic of thyroid epithelium, and that this step in morphogenesis makes possible the penetration and anastomosis of blood vessels at this time. It is evident that a considerable part of the enlargement of the gland from 7 to 10 days of incubation is due to the tremendous increase in the vascular channels within the thyroid gland, rather than to proliferation of thyroid epithelium. The initial steps in production of colloid and in formation of primary follicles occur during the stages when cords of epithelial cells are separated by prominent sinusoids, and it may be that this relationship favors the rearrangement of the epithelial cells into primary follicles. However, follicle formation does take place *in vitro* in the absence of such prominent vascular spaces (Gaillard, 1953; Tixier-Vidal, 1955). The transformation of the sinusoidal vascular pattern into the capillary pattern beginning at approximately 11 days of incubation is clearly correlated with the separation and growth of the individual follicles; it may be simply the consequence of the pressures and tensions exerted on the vascular spaces by increase in size and number of follicles. It is interesting to note that this transformation of the vascular pattern begins at the very stage when the thyrotrophic hormone of the anterior pituitary gland becomes essential for normal histogenesis of the thyroid gland (Martindale, 1941).

Hypophysectomized embryos. Hypophysectomy modifies histogenesis of the thyroid gland of chick embryos (Fugo, 1940; Martindale, 1941). No colloid is present in the gland until 13½ to 14 days of incubation. Colloid droplets then appear within the characteristic cords of cells and small underdeveloped follicles make their appearance. These never attain normal size and are scattered sparsely throughout the gland. After the eighteenth day such colloid as has formed begins to disappear, and is almost totally lacking by the end of incubation.

Development of the vascular pattern is similarly retarded and incomplete in thyroid glands of hypophysectomized embryos. By 11 and 12 days of incubation vasculogenesis of thyroid glands of hypophysectomized embryos lags behind that of control embryos (Figs. 9 and 10). Transformation of the prominent sinusoidal spaces into capillary-like vessels fails to take place in thyroid glands of hypophysectomized embryos (Figs. 12 and 14). This appears to be the

consequence of the incomplete transformation of the epithelial cords into follicles and of the failure of the follicles to increase in number and size in the absence of the pituitary. Both Fugo (1940) and Martindale (1941) mentioned the presence of large blood vessels within thyroid glands of hypophysectomized embryos; these are especially well illustrated by Fugo in his figures 10, 12, and 14. These illustrations are important since they demonstrate that these vascular spaces are just as large in his uninjected specimens as they are in the injected specimens of the present study, thereby eliminating any possibility that the vascular channels in the latter might be exaggerated due to abnormal pressures exerted at the time of injection.

Relation of Vasculogenesis to Functional Differentiation

The information which is available in the literature concerning functional differentiation in thyroid glands of normal chick embryos is summarized in Table I in order to facilitate comparison with changes in the vascular pattern.

Capacity to accumulate I^{131} selectively is already present in thyroid glands of 5-day embryos (Trunnell and Wade, 1955) prior to the time that blood vessels penetrate into the substance of the thyroid gland. Onset of this capacity is perhaps correlated with the establishment of a vascular plexus on the outer surface of this gland between 4 and 5 days of incubation. This selective accumulation is considerable by 7 days of incubation at which time the concentration of radioactive iodine in the thyroid gland averages 400 to 7700× its concentration in the blood; no organic binding of I^{131} has occurred as yet (Wollman and Zwilling, 1953). By this time a rather uniform network of blood vessels has been established throughout the thyroid glands, and the epithelium has subdivided into cords or plates of cells. As the diameters of the blood vessels increase markedly on the eighth, ninth and tenth days of incubation, the embryonic thyroid gains its capacity to bind I^{131} organically (Wollman and Zwilling, 1953). Monoiodotyrosine is first synthesized by 8½ days of incubation, diiodotyrosine by 9¼ days, and thyroxine by 9¾ days (Trunnell and Wade, 1955). By 10 days of incubation when the sinusoids have attained their maximal diameters thyroxine is present in the thyroid in easily detectable amounts (Trunnell and Wade, 1955).

The transformation of the sinusoid-like spaces into narrow capillary-like channels beginning on the eleventh day of incubation is

correlated with a sudden increase in I^{131} uptake between 10 and 13 days of incubation (Stoll and Blanquet, 1952a; Trunnell and Brayer, 1953) and with increased retention of accumulated I^{131} (Maraud, Stoll, Marcario, and Blanquet, 1954). These functional changes suggest an increased rate of synthesis of iodinated compounds.

Evidence concerning the time of onset of release of thyroxine into the blood stream is difficult to obtain. The only direct approach appears to be that of Meyniel, Blanquet, Mournier, Stoll, and Maraud (1955) who were able to demonstrate the presence of thyroxine tagged with I^{131} in the bodies of 10-day chick embryos from which the thyroid glands had been removed prior to analysis. Whether this means that thyroids of 10-day embryos have released small quantities of thyroxine or that small amounts are synthesized elsewhere in the body remains problematical.

Indirect evidence also indicates that thyroxine is released by 10 to 12 days of incubation. Body growth of embryos given thiourea at 7 days of incubation appears to be checked for the first time at 11 days of incubation (Vidal, 1952). Fugo (1940) noted that in hypophysectomized chick embryos developing feathers become melanotic by the twelfth day and that the condition can be corrected with the injection of thyrotrophin. Moreover, Bartels (1944) and Kraicziczek (1956a) described morphological changes in thyroid glands at 11 to 12 days which are suggestive of hormonal release. Thus it would seem that onset of release of thyroxine is correlated with onset of transformation of the sinusoidal pattern into the capillary vascular pattern.

Another critical period in the development of the thyroid appears to occur immediately prior to hatching. Kraicziczek (1956a) maintains that morphological features of 18-day thyroids indicate a turbulent outflow of secretion. Her observations are substantiated by the facts that at this time there is a fall in iodine (Sun, 1932) and thyroxine (Trunnell and Wade, 1955) content of the gland. That thyroxine is released in greater amounts toward the end of incubation is suggested by experiments in which synthesis of the hormone is blocked by administration of antithyroid drugs. Under these circumstances resorption of yolk, withdrawal of yolk sac, and initiating of hatching are blocked (Grossowicz, 1946; Adams and Bull, 1949; Vidal, 1952; Romanoff and Laufer, 1956). Such a block does not occur if both thyroxine and antithyroid drugs are administered (Gros-

sowicz, 1946). The vascular pattern has completed its transformation into a network of capillaries in normal embryos by the time this considerable outflow of thyroxine into the blood stream is postulated.

At the present time the only direct evidence available concerning functional differentiation of thyroid glands of hypophysectomized chick embryos (other than the fact that plumage of hypophysectomized embryos becomes melanotic and that hypophysectomized embryos fail to withdraw their yolk sacs and prepare for hatching) is that provided by the study of Maraud, Stoll and Blanquet (1957). They observed in hypophysectomized chick embryos a marked reduction in the thyroid gland's ability to concentrate I^{131} by 13 days of incubation (but not at 10 days). In addition, histological studies (Fugo, 1940; Martindale, 1941) indicate that the functional activity of thyroid glands of hypophysectomized chick embryos is greatly reduced by 11 days of incubation as shown by the small number and size of the follicles and by the delayed formation and small quantities of colloid. The vascular pattern in thyroid glands of hypophysectomized chick embryos is an additional indication of reduced functional activity since it fails to transform into the capillary network which is characteristic of the actively functioning thyroid glands of normal 18-day embryos.

Summary

1. The development of the vascular pattern within thyroid glands of normal and hypophysectomized chick embryos has been studied with the aid of celloidin serial sections of thyroid glands of embryos injected with India ink.

2. In normal embryos an external vascular plexus forms on thyroid glands between 4 and 5 days of incubation. Branch vessels from this plexus penetrate the glands during the sixth day of incubation and anastomose with one another to establish a uniform network by 7 days of incubation. Diameters of internal blood vessels increase progressively until 10 days of incubation. Beginning on the eleventh or twelfth day of incubation these sinusoid-like channels are transformed progressively into capillary-like vessels which form a network around each follicle. This transformation is complete by 18 days of incubation.

3. Initial steps in vasculogenesis in hypophysectomized embryos are similar to those in control embryos, but by 11 days vasculogenesis

in hypophysectomized embryos begins to lag behind that of control embryos of the same age. The vascular pattern in hypophysectomized embryos remains sinusoidal; it fails to transform into a network of capillaries.

4. Changes in the vascular pattern are intimately associated with changes in histogenesis. Increase in number and size of follicles in normal embryos appears to be responsible for the transformation of the vascular pattern from sinusoid-like spaces into a network of narrower capillary-like vessels. Failure of this transformation to occur in hypophysectomized embryos is correlated with the small number and small size of the follicles in the latter.

5. A number of changes in the vascular pattern can be correlated with known changes in functional activity of the gland as follows: a) establishment of an external vascular plexus—onset of capacity to accumulate I^{131} selectively (between 4 and 5 days); b) penetration of vessels into the gland and anastomosis to form a uniform internal network—increased capacity to accumulate I^{131} selectively (6 to 8 days); c) attainment of maximal diameters of internal vessels —attainment of capacity to bind I^{131} organically and to synthesize monoiodotyrosine, diiodotyrosine, and thyroxine (9 to 10 days); d) onset of transformation of sinusoid-like spaces into networks of capillaries—increased synthesis of iodinated compounds and initial release of thyroxine into the blood stream (11 to 12 days); e) attainment of definitive vascular pattern—release of considerable amounts of thyroxine into the circulation (16 to 18 days).

6. Failure of transformation of sinusoid-like spaces into a network of capillaries in thyroids of hypophysectomized embryos is probably correlated with inability to synthesize increased amounts of thyroxine and with the release of subnormal quantities of thyroxine into the blood.

7. Changes in vascular patterns of thyroid glands of chick embryos seem to be reliable indices of changes in functional activity to the extent that the latter are now known.

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LACK OF GLYCOPROTEIN RESPONSE IN IRRADIATED CHICKEN EMBRYOS

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POLYSACCHARIDE associated with serum proteins has been found to be elevated in several clinical conditions including neoplastic disease, rheumatoid arthritis, tuberculosis, various infections and pregnancy (1-4). It has also been observed in the adult rat that increases in plasma polysaccharide take place by the 6th day following 700 r of whole body x-irradiation and remain elevated until about the 12th day post-irradiation (5). Shetlar et al. (2) have intimated that increases in plasma polysaccharides under clinical conditions are related to tissue proliferation or repair.

It is of interest to test the serum polysaccharide response in various stages of chicken embryos and newly hatched chicks for 2 main reasons: (1) The above-mentioned polysaccharide reaction to irradiation may be essentially lacking in the very young animal; (2) The normal course of increase of protein-bound polysaccharide (a doubling in concentration of both glycoprotein and seromucoid takes place at hatching) might, conversely, be inhibited by x-irradiation.

MATERIALS AND METHODS

Because of the relatively rapid metabolism and syntheses taking place in these growing embryos, most of the serum samples were taken three days post-irradiation. It is after this interval post-irradiation that the most drastic derangements in serum lipoproteins have been detected (6). Thus the conditions under which Schacter et al. (5) observed increases in serum glycoproteins in the adult rat were probably relatively closely met. In order to ensure, however, that no acute changes in serum polysaccharide would escape detection,

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some embryos and chicks were also bled at 1, 2, and 7 days following exposure. Twelve- and 13-week-old capons were bled 4 and 7 days post-irradiation respectively.

For each experiment involving embryos, blood was pooled from at least twenty-four animals. In the case of newly hatched chicks and of 4-day-old chicks, blood was pooled from twelve to fifteen birds. Serum was obtained by centrifuging the blood approximately one hour after clotting. It was then frozen and stored for analysis.

Five hundred roentgens of x-irradiation were given to the embryos. Newly hatched chicks and 12-week-old capons received 600 roentgens. The radiation factors were 250 K.v.p., 15 ma, 35 cm T.O.D., 0.21 mm Cu inherent, plus 0.5 mm parabolic Cu, plus 1.0 mm Al filters, HVL = 1.9 mm Cu center of parabolic filter. Roentgens were measured in air.

Total serum glycoprotein[1] was determined by the method of Lustig and Langer (7) as modified by Weimer and Moshin (8). The total seromucoid fraction[2] was determined by the procedure of Winzler et al. (9) as modified by Weimer and Moshin (8). All analyses were carried out in duplicate.

Results and Discussion

No significant changes in serum glycoproteins or seromucoids were detected in younger animals as a result of irradiation. On the other hand, significant increases of both total serum polysaccharide and the seromucoid fraction were observed in irradiated 12-week-old capons (Table I).[3]

As reported earlier (10), during the course of normal development, both serum glycoproteins and seromucoid fraction are present in low concentration from the 10th to the 20th day of incubation. However, between the 20th and 22nd day, during the period of hatching, a

[1] The non-glucosamine polysaccharide component or hexose moiety of the serum glycoproteins precipitated by an ethanol concentration of 90 per cent by volume at 25°.

[2] The non-glucosamine polysaccharide component or hexose moiety of the perchloric acid-soluble, phosphotungstic acid-insoluble proteins occurring in serum.

[3] A hemoconcentration amounting to approximately 25 per cent (based on relative total serum protein) was observed in the irradiated capons. Correction was made for this hemoconcentration before values for relative differences in glycoprotein and seromucoid values between irradiated and control capons were placed in Table I. Aside from the hemoconcentration, older birds displayed other symptoms of x-irradiation, such as poor appetite and general listlessness, to a greater extent than did the younger chicks receiving the same 600r dose of x-irradiation. A subsequent experiment in which the dose was reduced to 500r provoked a response in only certain capons by 7 days post-irradiation (Table I, last entry).

TABLE I

Age Group	Total Serum Polysaccharide		Seromucoid Polysaccharide	
	Control mean mg. per 100 ml	Irradiated mean mg. per 100 ml	Control mean mg. per 100 ml	Irradiated mean mg. per 100 ml
13 days incubation	17 ± 1.0**	17 ± 1.6	3 ± 0.3	3 ± 0.5
14* days incubation	15 ± 1.1	17 ± 0.8	3 ± 0.2	3 ± 0.3
15* days incubation	19 ± 1.3	21 ± 1.4	3 ± 0.4	4 ± 0.4
16* days incubation	20 ± 1.0	19 ± 1.5	5 ± 0.5	4 ± 0.6
18 days incubation	26 ± 2.2	26 ± 2.8	4 ± 0.8	5 ± 0.3
21 days incubation	41 ± 1.4	40 ± 2.0	9 ± 1.4	9 ± 1.7
1* day post hatching	65 ± 2.3	65 ± 3.6	11 ± 0.6	12 ± 1.7
2* days post hatching	70 ± 2.9	69 ± 2.5	11 ± 1.5	12 ± 1.5
3* days post hatching	76 ± 3.6	76 ± 5.1	13 ± 1.1	13 ± 1.0
7* days post hatching	61 ± 1.2	55 ± 1.1	11 ± 0.5	11 ± 0
12* wks. (Capon) (600r)	109 ± 7.3	139 ± 6.2	22 ± 1.6	40 ± 6.4
13 wks. (Capon) (500r)	90 ± 4.6	92 ± 8.3	17 ± 3.1	20 ± 3.2

* 14, 15 and 16 day embryos irradiated on 13th day. 1, 2, 3 and 7 day-old chicks irradiated on day of hatching. Capons irradiated 4 and 7 days prior to bleeding. All others irradiated 3 days prior to bleeding.

** Standard deviation.

doubling in concentration of both of these moieties takes place in the serum. This increase is correlated with the fact that growth of both the liver and the whole body ceases abruptly at this time (10). It seems especially noteworthy that irradiation given prior to hatching had no detectable influence on the normal increase of serum glycoproteins and seromucoids. In contrast, dense lipoproteins (— s 0—10) which also normally appear in double concentration in the serum after hatching, are observed to be sharply reduced following the animal's exposure to 500 r of whole body x-irradiation (6).

Previous studies in this laboratory have shown that serum albumin is significantly reduced in the developing chicken by 3 days post-irradiation (6). Utilizing the rat as an experimental animal, Shacter et al. have correlated this reduction of serum albumin with a decrease in total serum sulfhydryl (5). They have further related the fall in sulfhydryl with an increase in serum glycoproteins. It is of interest to note that these relationships do not exist in the embryo chicken. The drop in serum albumin (and hence presumably sulfhydryl) is not followed by an increase in glycoproteins or seromucoid.

It is possible that the embryo's failure to increase its serum glycoproteins following irradiation is due to its limited mechanisms with respect to the synthesis of these moieties. On the other hand, the embryo may lack homeostatic regulatory mechanisms present in the adult.

Conclusions and Summary

From the above results it would appear that embryo chickens and very young chicks are incapable of giving a strong serum polysaccharide response when irradiation is employed as the stimulating agent. The cells and mechanisms for placing these moieties into the serum are, however, sufficiently well developed and radio-resistant so that the normal increases in the serum are relatively slightly altered by 500-600 roentgens.

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MATERNAL ENVIRONMENT AND FETAL DEVELOPMENT IN THE PIG[1,2,3,4,5]

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THE mammalian fetus presents certain characteristics not found to the same degree in adult organisms. First, mammalian fetuses grow rapidly. Such growth involves at least two uniquely distinct mechanisms, cellular division and increase in cellular volume. Secondly, they are rapidly differentiating; differentiation takes place both at the intra- and extra-cellular levels. Thus, there is a considerable difference in complexity and probably, also, in total and partial reactivity to nutritional deficiencies as a function of age.

Elucidation of the growth pattern during fetal life and the manner in which it is modified by nutrition is desirable for a number of reasons. Differences between the rate and order of development of component parts can be expected to be associated with the development of function and are likely to throw light on problems of fetal physiology. Again, information regarding the normal range of variation in size, and the proportions which the various organs normally bear to each other at different stages of development, is needed. A standard may be available against which supposedly abnormal specimens can

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be judged, and the effect of experimental treatments assessed. Thirdly, if certain organs whose growth or relations are little affected by variations in nutrition could be found, this could provide a useful guide for determining the ages of fetuses when these are not known.

To study the influence of nutrition on pre-natal development of pigs it is essential to have reliable information of the normal course of growth of the fetus and of its component parts and tissues at different ages. Information on these details is notably lacking except for the work of Lowrey (1911) and Wallace (1948).

This investigation was carried out in an attempt to trace the course of the prenatal development and the relative growth of the various organs with particular reference to uterine environment and maternal nutrition.

Materials and Methods

Breeding Stock:

The foundation stock for the study was built by crossing five Danish Landrace boars each with five relatively unrelated groups of five Chester White sows each. The progeny were divided equally and at random at weaning (56 days) into two lines—one group was full-fed (high) from weaning to 150 lbs.; the other group was fed 70 per cent as much as the high-plane pigs ate at comparable weights up to 150 lbs. From 150 lbs. in weight until farrowing, all pigs were hand fed to prevent the high plane pigs from becoming obese. The high-plane animals received sufficient feed to allow them to gain approximately 1.5 to 2.0 lbs. daily. The low-plane pigs received 70 per cent of this amount. Following farrowing, the high-plane pigs were again full-fed, and the low-plane sows received 70 per cent of full feed.

This system was maintained for ten generations throughout four years. The lines have been maintained as closed populations throughout, with inbreeding being kept at a minimum by systematic crossing of the groups within the lines. The same system of mating was practiced in each line so as to keep the inbreeding coefficient as nearly alike as possible.

Experimental Animals:

Thirty Palouse gilts representing the two planes of nutrition (high and low) in F_{10} were maintained in dry lot throughout the study and the same rations were fed to both high and low plane pigs. The rations were calculated to provide adequate protein, minerals and

vitamins at the level fed to pigs of low-plane, the only deficiency being energy.

The gilts were checked daily for estrus with vasectomized boars. At the third estrous cycle after puberty the gilts were mated to fertile boars. The gilts in each of the two planes of nutrition were assigned at random to be slaughtered 38, 69 or 100 days post-coitum.

Dissection Technique:

The genital tract of each gilt was removed immediately after slaughter and the gravid uterus was cut open from the tubal end. The following values were recorded for each individual fetus in sequence in the uterine horn at 38 days: sex and body weight, volume of amniotic and allantoic fluid and weight of the placental membranes. The umbilical cord of the fetus was tied near the navel before separation from the placentae.

Thirty 69-day fetuses, eighty-four 100-day fetuses of both sexes and ten adult gilts were carefully dissected (Table 1). The following

TABLE 1
EXPERIMENTAL GROUPS OF FETUSES AND ADULT ANIMALS WHICH WERE DISSECTED

Age at dissection	Low		High	
	Females	Males	Females	Males
Fetus (38 days)	51		30	
Fetus (69 days)	11	19	—	—
Fetus (100 days)	27	29	13	15
At birth	Five males at full term			
Adult gilts	Ten gilts of an age of		to	days

glands and organs were dissected from the adjacent connective tissues and weighed: pituitary, thyroid, adrenals, gonads, digestive tract (full), liver, spleen, kidneys, lungs and trachea, heart and dressed carcass. Carcasses of 100-day fetuses were frozen after dissection; a cross section of the body was made at the 10th rib and the surface area of *longissimus dorsi* muscle was traced on acetate paper. The surface area of the *longissimus dorsi* muscle was also traced in the carcasses (cooled) adult gilts at the 10th rib.

Degenerating and non-viable fetuses were not considered and were excluded from the data.

Microscopic Examination:

Eighty-one 38-day fetuses (of undetermined sex) on the two planes of nutrition were used to study the prenatal ossification pattern. The connective tissues were cleared and the skeletal system was stained. The length and thickness of the following ossified bones were measured microscopically using a calibrated micrometric eye piece divided into 1/10 mm: 1. Mandible, 2. Scapula, 3. Tenth rib, 4. Ilium, 5. Femur, 6. Tibia, 7. Fibula, 8. Humerus, 9. Ulna and 10. Radius (Figures 1 and 2). The number of ossified ribs and ossified vertebrae (body and process) was also recorded as judged microscopically.

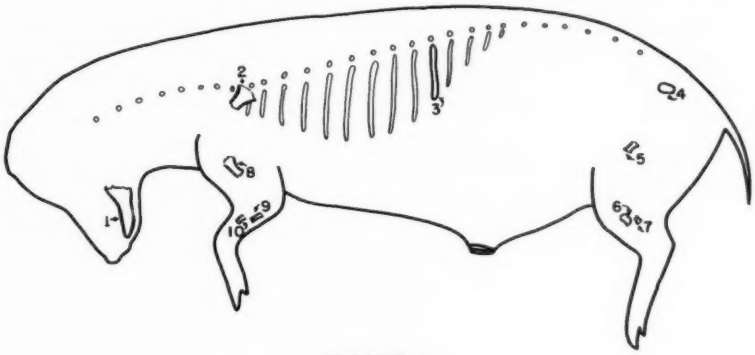

FIGURE 1
The ossification centers as they appeared in the 38-day fetuses.

Tissue Clearing Technique:

The fetuses were fixed in 10% formalin and cleared in the KOH solution until the skeletal elements were clearly visible. The fetuses were then selectively stained; the ossified tissue with Alizarin Red S (1: 10,000 in 2% KOH). The specimens were rinsed in several changes of distilled water over a period of several hours to rinse out the KOH solution and the excess stain. The fetuses were gradually dehydrated through a series of increasing concentrations of glycerol (1/4 glycerol:3/4 H_2O; 1/2 glycerol:1/2 H_2O; 3/4 glycerol:1/4 H_2O; 1 glycerol) and then stored in a second, or even third, change of glycerol. This gradual dehydration was necessary to prevent shrinkage and resulting deformity of body shape. The specimens were turned every few hours to insure even clearing on all sides. Great care was

taken to handle the fragile jelly-like fetuses by supporting both the head and body with rubber-gloved hands.

On computing the mean average of the prenatal development, an average was calculated for each litter. The mean average of the experimental groups was based on the average of each sow. The data were examined statistically by the analysis of variance techniques (Snedecor, 1950).

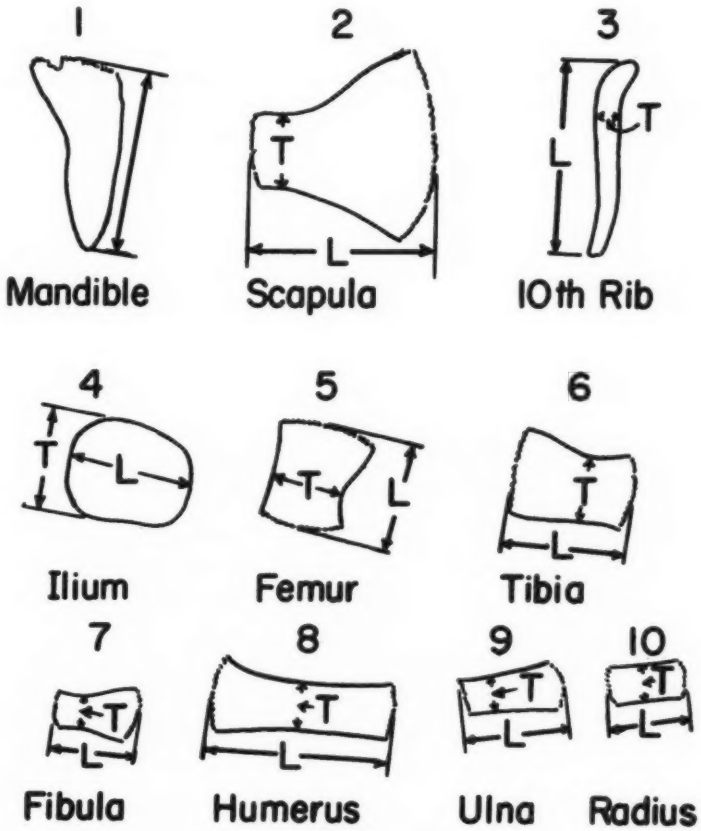

FIGURE 2
Procedure for microscopic measurements of ossification centers in the embryos.

Results and Discussion

Effect of Maternal Nutrition

The effect of plane of nutrition on the prenatal development of skeletal system was studied in 38-day fetuses. At this stage, the clearing of embryonic tissues other than skeleton by KOH is possible. The effects of plane of nutrition and of fetal sex on fetal organ development were studied in 100-day fetuses. At this stage the fetal organs would have been developed to a measurable stage.

Fetal Skeletal System:

At 38-days post coitum the different anatomical parts of the skeletal system were differentiated. The ossification centers were stained with Alizarin Red (Figure 3). The plane of nutrition for ten successive generations had no significant effect on the length or thickness of ossified portions in all parts of the fetal skeletal system (Table 2). However, there were significant differences between litters. This is partly explained by the fact that all the pregnant sows were slaughtered 38-days post-coitum. In the sow the duration of the estrous period varies from 1 to 5 days with an average of 2 days, and ovula-

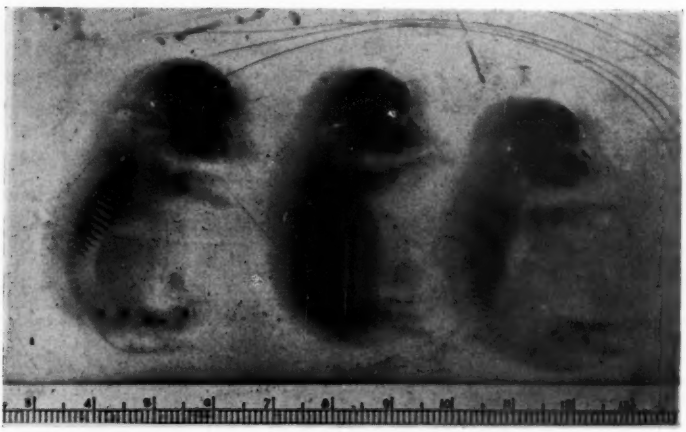

FIGURE 3

Three 38-day fetuses after tissue clearing, showing uniformity of ossification centers. The fetus on the right is from the Low plane group and the two fetuses on the left are from the High plane. (Photographed in glycerol medium).

tion takes place more nearly in the middle of the heat period. There is also variation in the "age of the ova" since they are not ovulated simultaneously. Consequently, the time of fertilization (onset of conception) varies among sows. It is also possible that differences in ossification centers within litters are due to differences in the uterine environment such as the uterine milk or the uterine temperature. Litter mates are only 50% related on the average; therefore, the genetic influence must be considered, even at this early stage, as making its contribution to the variation within litters.

This is an evidence that skeletal growth of the fetus is very regular. Some dimensions increase much more rapidly than others, with the result that the body proportions change. In sheep, the skull develops

TABLE 2
EFFECT OF MATERNAL NUTRITION ON SKELETAL DEVELOPMENT
(38-day embryos)

Skeletal measurement (mm.)		LL Range	LL Mean	HH Range	HH Mean	Between Planes	Between Litters
Mandible	L	4.498-7.712	5.857	5.017-8.635	6.00	NSD	***
Scapula	T	1.238-1.582	1.413	1.350-1.695	1.478	NSD	**
	L	1.350-2.825	2.044	1.350-2.938	1.918	NSD	***
10th Rib	T	0.308-0.364	0.339	0.306-0.392	0.347	NSD	**
	L	2.595-5.190	3.923	2.712-4.844	3.602	NSD	**
Ilium	T	0.308-0.847	0.632	0.448-0.792	0.683	NSD	***
	L	0.452-1.125	0.703	0.560-1.130	0.680	NSD	***
Femur	T	0.504-0.840	0.689	0.560-0.791	0.679	NSD	***
	L	0.336-1.176	0.687	0.280-0.952	0.551	NSD	***
Tibia	T	0.392-0.896	0.587	0.448-0.728	0.563	NSD	**
	L	0.336-1.064	0.621	0.280-0.728	0.483	*	***
Fibula	T	0.336-0.563	0.394	0.280-0.560	0.323	NSD	**
	L	0.224-0.672	0.414	0.224-0.728	0.287	NSD	***
Humerus	T	0.616-1.120	0.798	0.672-1.064	0.758	NSD	***
	L	0.616-1.848	1.223	0.728-1.624	1.073	NSD	***
Ulna	T	0.420-0.619	0.487	0.392-0.560	0.490	NSD	***
	L	0.616-1.400	0.980	0.616-1.120	0.881	NSD	***
Radius	T	0.392-0.504	0.438	0.392-0.504	0.426	NSD	***
	L	0.672-1.512	1.074	0.672-1.288	0.994	NSD	***
No. of Ossified Ribs		13-16	14	13-16	14	NSD	***
Tranverse process		18-32	28	14-33	25	NSD	***
Body process		20-33	28	20-32	28	NSD	***

* Significant at 10% level
** Significant at 5% level
*** Significant at 1% level
NSD No significant difference
L Bone length
T Bone thickness

to a relatively lesser degree during fetal life than the mandibles, and skull measurements indicate the cranial portion to be earlier maturing than the facial (Joubert, 1956). Also, the anterior cervical vertebrae are earlier maturing than those of the posterior extremity (caudal vertebrae).

Fetal Organs:

The plane of nutrition of previous and present generations had no significant effect on the weight of the fetal organ 100-days post coitum (Table 4). There were significant differences between litters in respect to the weight of the following: fetus, placental membranes, spleen, digestive tract, kidneys, heart, dressed carcass and liver, also the length of the umbilical cord. Also, the weights of the fetus and the dressed carcass (100-day fetuses) were not affected by sequence in uterine horn (Figures 4, 5, 6).

The 100-day fetuses were frozen and a cross-section was made at the 10th rib. The surface area of the *longissimus dorsi* averaged 0.14 square inches. The development of the fetal *longissimus dorsi* was not affected by plane of nutrition or sequence in the uterine horn

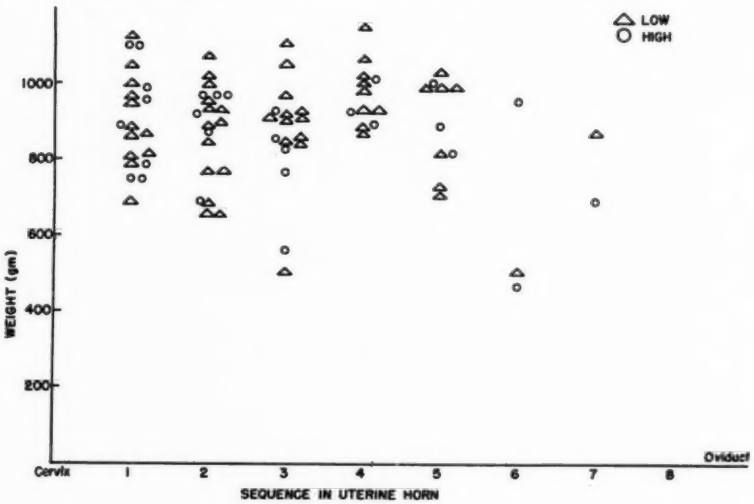

FIGURE 4
The weight of 100-day fetuses in relation to sequence in the uterine horn.

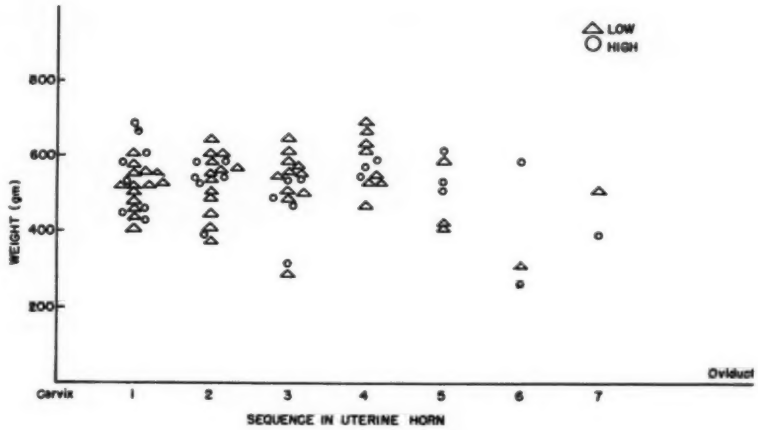

FIGURE 5

The weight of dressed carcass (with limbs and without head) in relation to sequence in uterine horn.

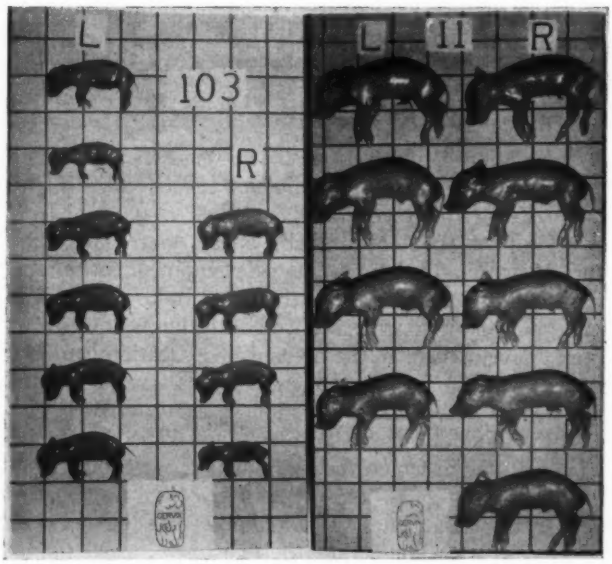

FIGURE 6

Fetuses of two gilts arranged according to sequence in uterine horn (L—left, R—right). Gilt number 103 was slaughtered 69-days post coitum. Gilt number 11 was slaughtered 100-days post coitum. The rate of fetal development was not associated to the sequence in the uterine horn or the number of fetuses per horn.

(Figure 7). The ratio between the surface area of the cross-section of the *longissimus dorsi* of the fetus to that of the adult (300 days of age) was 1:37.

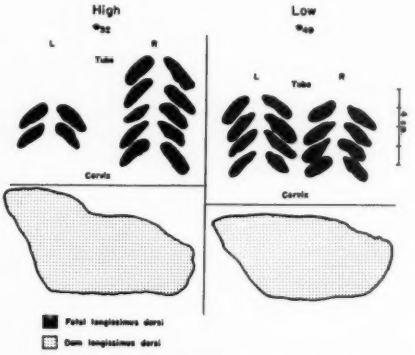

FIGURE 7

The relationship between the surface area of *longissimus dorsi* at the 10th rib in the 100-day fetus and the adult gilt 300 days of age. Top: Cross sections taken from fetuses of two gilts. The fetuses are arranged in sequence in the two uterine horns (L—left horn, R—right horn). Each fetus is represented by two cross sections, the left and right muscle. Note that the development of fetal *longissimus dorsi* is not related to sequence in uterine horn or the plane of nutrition. Bottom: Cross section taken from the left side of the adult gilts. The surface area is on an average thirty-seven times as much as the fetal muscle.

In rats, reduction of the food intake to one-half to one-third or the absence of protein from the diet did not result in a decrease in the number and weights of placentae (Campbell *et al.*, 1953). This apparently is brought about by drawing on the maternal reserves.

On the other hand, the prenatal development of lambs is profoundly affected by the level of nutrition of the ewe during the latter part of pregnancy (Wallace, 1948). The insignificant effect of nutrition on fetal development in the present investigation can be explained on a genetical basis. The experimental animals were selected for ten successive generations using a selection index: $I = B + 2W + 35G$, where B is the number of pigs born alive and W is the number of pigs weaned in the litter in which the individual pig was born and G is the average rate of daily gain. This type of selection was carried out for both the Low and High lines. It is possible that the same genes were being selected in both lines; thus the two lines caused the diminished effect of nutrition on prenatal growth.

There is adequate evidence to support the observation that the rate of fetal growth is affected by genetical factors. In Chester White and Poland China pigs, the purebred fetuses are significantly heavier than crossbred fetuses at 25 days post-coitum. A similar but statistically insignificant trend was found at the 70th day (Baker *et al.*, 1958). Cattle show breed differences, particularly between Holsteins and Jerseys, in fetal measurements (Swett *et al.*, 1948).

Individual differences in maternal growth and fatness have no effect on embryo or fetal development, as shown by path coefficient analysis (Waldorf *et al.*, 1958). It has been suggested that the total energies available at one time must comprise a closed system and that for these available energies, somatic growth (cellular increase) and integrative growth (activity) must compete (Collins, 1953).

Fetal Endocrine System:

The weight of the thyroid, adrenals and pituitary of the pig fetus 100 days post coitum was not affected by the plane of nutrition (Tables 3 and 4). There were significant differences within litters in the weight of the pituitary gland and adrenals, but not in the thyroid. The differences between sexes were more pronounced on the weight of the pituitary gland, than on the thyroid or adrenals. This may be considered as an evidence for function of fetal endocrine system. The decapitation of the rat and rabbit fetuses, respectively, leads to an inhibition of growth of the adrenal with some modification in cortical cells, whereas similar decapitation accompanied by injections of an adrenocorticotrophic preparation prevented the appearance of such adrenal changes (Wells, 1948 and Jost, 1953). Moreover, since the elimination of fetal hypophysial secretions, late in pregnancy, also resulted in the failure of the fetal gonads to develop normally; this was taken as evidence for gonadotropic function at this stage of development (Jost, 1954).

Effect of Sex

At 69 days post coitum, the effect of sex was significant on the weight of fetus and on the placental membranes but not on the placental fluids (Table 5). At 100 days post coitum there were no significant sex differences in the weight of fetus, placental membranes or placental fluids. In humans, males are heavier than females at a given weight of placenta (McKeown and Record, 1953). Such dif-

TABLE 3
ORGAN DEVELOPMENT OF 100-DAY FETUSES SECURED FROM SOWS ON LOW OR HIGH PLANES OF NUTRITION

Item	Low* ♂ Range	Low* ♂ Mean	Low* ♀ Range	Low* ♀ Mean	High** ♂ Range	High** ♂ Mean	High** ♀ Range	High** ♀ Mean
Embryo weight	515-1130	933.6	666-1034	854.3	692-1008	893.3	468-1110	840.1
Fluids	34-481	171.7	70-300	159.3	80-340	181.7	44-300	164.9
Membranes	113-369	235.9	144-388	220.6	119-329	218.3	144-353	210.8
Thyroid	.14-.38	0.22	.13-.38	0.23	.15-.32	0.23	.13-.31	0.24
Adrenals	.05-.26	0.14	.08-.20	0.13	.08-.15	0.12	.09-.19	0.13
Pituitary	.02-.05	0.035	.01-.06	0.032	.02-.04	0.028	.02-.04	0.030
Spleen	1.2-2.9	1.86	1.0-3.0	1.90	1.2-1.9	1.56	1.0-2.1	1.61
Digestive tract	32.6-81.0	58.7	38.2-71.0	55.5	43.2-70.7	56.1	29.1-70.6	51.3
Genital organ	.19-.54	0.33	.03-.26	0.12	.21-.40	0.29	.11-.19	0.15
Kidney	5.9-13.0	9.14	6.4-13.2	8.64	6.6-10.4	8.49	5.3-11.9	8.88
Lung & trachea	15.6-39.0	29.3	9.4-39.0	26.8	21.4-41.5	29.8	13.8-36.9	26.9
Heart	5.1-11.5	7.99	6.0-10.2	7.59	6.3-9.7	7.53	4.3-10.4	7.48
Dressed carcass	289-680	553.9	375-691	517.4	394-613	539.6	260-694	503.6
Umbilical cord	20-36	28.6	21-38	29.7	19-30	25.7	20-35	23.6
Liver	16.0-42.3	29.9	20.5-37.2	26.9	23.9-34.1	27.8	13.4-39.3	26.3

* LL and HL
** HH and LH

ferences between fetal growth rate are determined by the endometrial environment. It may be explained largely, though not entirely, by differences in placental weight.

The testis was significantly heavier than the ovary in the 100-day fetus (Table 4). At the same stage there were no significant sex differences in the weight of internal organs. In humans, the fetal viscera appear as a rule to be relatively heavier in the females (excepting the thymus, lungs, and kidneys) than in the males (Jackson, 1909).

TABLE 4
ANALYSIS OF VARIANCE FOR THE DIFFERENCES BETWEEN NUTRITION, LITTER, AND SEXES (100-DAY FETUSES)

Item	Planes	Litters	Sexes
Fetus (gm.)	NSD	*	NSD
Placental fluids (ml.)	NSD	NSD	NSD
Membrane (gm.)	NSD	***	NSD
Thyroid	NSD	NSD	NSD
Adrenals	NSD	***	NSD
Pituitary	NSD	*	***
Spleen	NSD	**	NSD
Digestive tract	NSD	**	NSD
Genital organs	NSD	NSD	***
Kidney	NSD	***	NSD
Lung and trachea	NSD	NSD	NSD
Heart	NSD	***	NSD
Dressed carcass	NSD	**	NSD
Umbilical cord	NSD	***	NSD
Liver	NSD	**	NSD

NSD No significant difference
* Significant at 10% level
** Significant at 5% level
*** Significant at 1% level

TABLE 5
EFFECT OF SEX ON PRENATAL DEVELOPMENT AND PLACENTATION

Items	Stage of pregnancy (days)	Females Range	Females Mean	Males Range	Males Mean	Statistical significance
Fetus weight	69	177-302	232.00	171-334	266.95	*
(gm.)	100	468-1110	849.65	515-1130	919.89	NSD
Placental weight	69	119-241	180.73	177-369	242.68	**
(gm.)	100	144-388	217.40	113-369	229.89	NSD
Volume of placental	69	200-640	328.18	80-580	295.00	NSD
fluids (ml.)	100	44-300	161.10	34-481	175.09	NSD

* Significant at 5% level
** Significant at 1% level

Fetal Sex Ratio:

The male:female ratio at 100 days of pregnancy varied from 4:0 to 1:7 with an overall average of 1:1. Also, there was no uniform distribution of both sexes in the uterine horn (Figure 8). The fetal sex ratio appeared to bear no relation to litter size. Litters in which the sex ratio was equal or almost equal predominated. Perry (1956) has reported that the fetal sex ratio appears to bear no relation to the ordinal number of the litter or to its size.

Prenatal Developmental Changes

The developmental changes of organ weight in relation to total body weight were studied in the prenatal life (69 to 100 days post

FIGURE 8
The fetal sex ratio in 100-day fetuses bears no relation to litter size.

coitum). This was compared with the proportion of organ weight at birth and in the mature pig. This percentage of digestive tract to total body weight was 4.18 in the 69-day fetus; this percentage increased until birth but it declined towards maturity (Table 6). The percentage of the other organs declined steadily. The carcass dressing percentage ranged from 60.56 to 62.24 in the prenatal life; dressing percentage was 64.72 at birth and 73.24 at maturity.

The weights of organs at 100 days post coitum and at birth are expressed as a percentage of those at 69 days post coitum (Figure 9). In the last 15 days before birth the relative growths of digestive tract, testis and adrenals were much higher than those of heart, spleen, liver, pituitary, lungs and thyroid. This demonstrates the differential growth rates of different organs prior to birth, presumably as a result of vital necessity of these organs at birth.

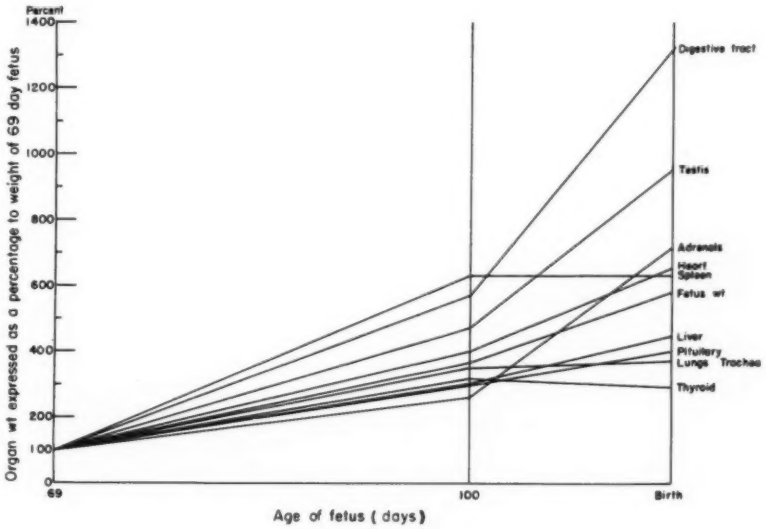

FIGURE 9

Developmental changes in the organ weight in the prenatal life. The weights of organs at 100-days post-coitum and at birth are expressed as a percentage of those at 69 days post-coitum. In the last two weeks of pregnancy, relative growth is much higher in digestive tract and testes than in liver and lungs.

TABLE 6
DEVELOPMENTAL CHANGES IN BODY ORGANS (REARED ON A LOW PLANE OF NUTRITION)

Items	Stage of development	Absolute Weights (gm.) 60-day fetus	100-day fetus*	At birth	Adult (gm)**	% Body Weight 69-day fetus	100-day fetus	At birth	Adult**
Wt. of fetus (or animal)		232.0	854.3	1352	142556	—	—	—	—
Endocrine Glands	Thyroid	.07	0.23	0.30	0.82	0.03	0.03	0.02	N
	Adrenals	.05	0.13	0.36	0.82	0.02	0.02	0.03	N
	Pituitary	.01	0.03	0.04	0.0454	0.004	0.003	N	N
	Testis	.07	0.33	0.67	—	0.03	0.038	0.05	N
	Ovary	.06	0.12	—	1.32	0.03	0.014	N	N
Digestive tract (bull)		9.7	55.5	128.0	7314	4.18	6.50	9.47	5.13
Liver		8.8	26.9	38.9	1948	3.79	3.15	2.88	1.37
Spleen		.03	1.9	1.9	177.0	0.01	0.22	0.14	0.12
Kidneys		3.0	8.6	11.6	3360	1.29	1.01	0.64	0.24
Lungs and trachea		7.7	26.8	28.9	1285	3.32	3.14	2.55	0.90
Heart		1.9	7.6	12.4	3768	0.82	0.89	0.56	0.26
Dressed carcass		144.4	517.4	875.0	104420	62.24	60.56	64.72	73.24

* Average for female
** Average for three 100-day LL sows
N Negligible

Placentation:

The relative weight of the placental membranes decreased slightly with advancing pregnancy (Figure 10). The relative growth of placental fluids was markedly decreasing while the relative weight of the fetus was increasing. The weight of placental membranes and the volume of placental fluids were not affected by the sequence in the uterine horn (Figures 11, 12).

General Discussion

Though growth of the individual from the time of fertilization until birth is continuous, it is possible to divide the pre-natal era into three periods upon the basis of certain more critical phases during life *in utero,* and the relative degree of development attained. The 'period of the ovum' lasts from the time of ovulation until attachment of the blastocyst to the endometrium. During the 'embryonic period,' the

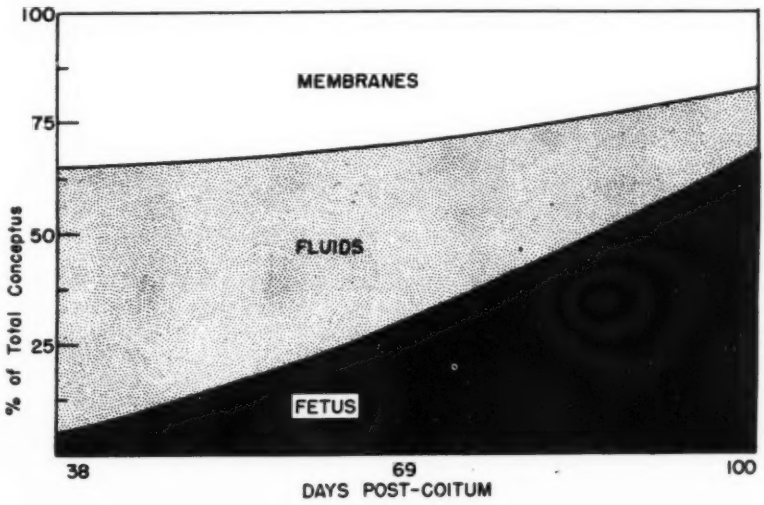

FIGURE 10

Developmental changes in the components of the conceptus from 38 to 100 days of pregnancy. The relative weight of placental membranes is slightly decreasing. The relative weight of the fetus is markedly increasing while the placental fluids are markedly decreasing.

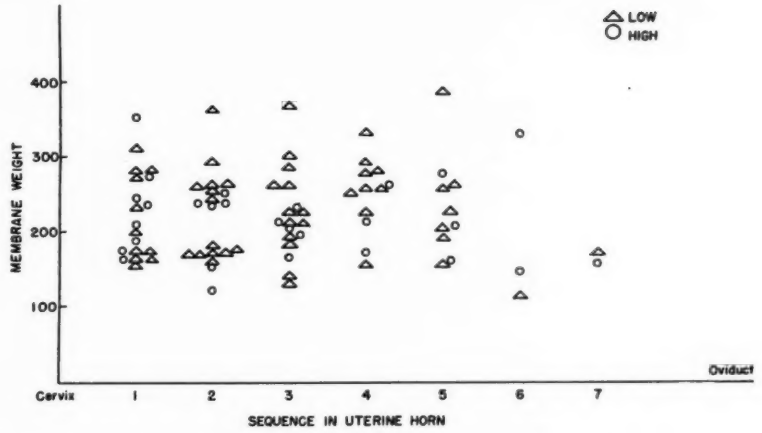

FIGURE 11
The weight of the placental membranes of individual fetuses in relation to sequence in uterine horns (100 days post-coitum).

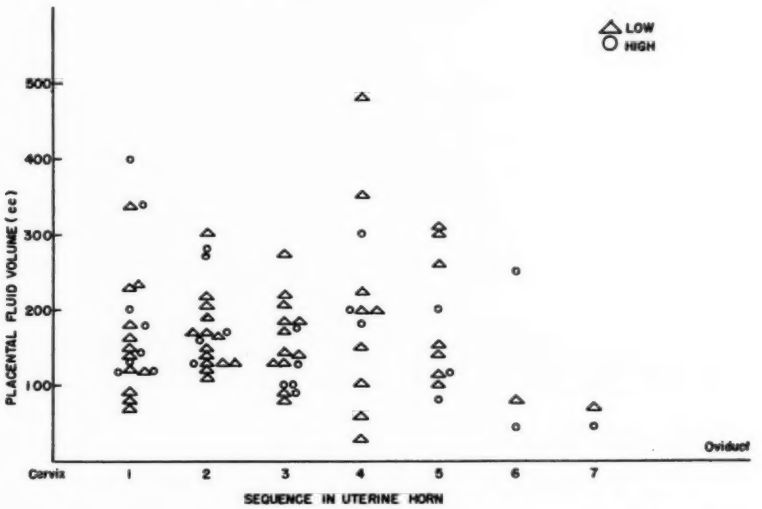

FIGURE 12
The volume of allantoic and amniotic fluids of individual fetuses in relation to sequence in the uterine horns (100 days post-coitum).

main organs and their systems are formed. The 'fetal period' terminates at birth.

The hypothesis advanced by Hammond (1952) is of great importance to the present problem. He suggests that during pregnancy, fetal and maternal tissues compete with one another for available nutrients, and that when the supply is limited fetal tissues are the more successful, owing to their higher metabolic rate. He further suggests that competition is not merely between fetus and mother; it also exists between, and within, the component tissues of each. Nerve tissue has first call upon nutrients, followed by bone, muscle and fat in that order of priority.

If certain nutritive regimes have the effect of slowing or increasing the growth rate of some parts more than others, then the manner in which the weight increment made by the fetus during any period is distributed depends upon the extent to which the nutritive conditions then prevailing are limiting the growth rate. In brief, the partition of available nutrients will vary with the level of nutrition.

The nutrition of the fetus at all stages, and especially at the initial stage, is determined to a considerable extent by the physiological activity of the embryo itself. The role of maternal organisms in providing the embryo with all necessary conditions for its normal development, though very important, is not decisive.

Among the organs which, as a whole, are well developed at the time of birth, differences in respect to their manner of growth appear to be to a large extent related to functional necessity. The way by which advanced development at term is achieved may differ in the case of individual organs; for instance, the liver and heart, both of which are presumably of functional importance in fetal life, make a high proportion of their growth in the earliest stages, while other, such as the digestive tract, not vitally important before birth, make a far higher proportion of their growth in the later stages.

In the components of the skeletal system where variations in function are not likely to be of importance in fetal life; differences between the parts in respect to rate and time of growth seem to depend largely upon their relative position. In the normal course of development and differentiation the embryo follows an anterior to posterior direction. Therefore, except where this order is upset by specific functional adaptations, anterior parts are older than posterior ones, developmentally speaking. Further, as the limb buds arise, the course

of development within them is in the proximodistal direction (Hamilton *et al.*, 1945).

Summary

Pigs were reared on two planes of nutrition (high and low) for ten successive generations. Gilts of F_{10} were slaughtered 38, 69 and 100 days post-coitum. The ossification pattern was studied in eighty-one 38-day fetuses using Alizarin Red S technique. Thirty 69-day and eighty-four 100-day fetuses were completely dissected.

1. a. The plane of nutrition for ten successive generations had no significant effect on the length or thickness of ossified portions in all anatomical parts of the fetal skeletal system. This may be related to the homozygosity achieved throughout the course of selection.
   b. There were differences in dimensions of ossification centers between litters, partly as a result of differences in time of fertilization in relation to duration of estrus.
2. a. Fetal organ development: The plane of nutrition had no significant effect on the fetal organs weight 100-days post coitum. There were significant differences between litters in respect to the weight of the following: fetus, placental membranes, endocrine glands, spleen, digestive tract, kidneys, heart, dressed carcass and liver and length of umbilical cord.
   b. The development of the fetal *longissimus dorsi* was not affected by the plane of nutrition or sequence in the uterine horn.
3. The effect of sex of the fetus was more pronounced on the pituitary weight than on the thyroid and adrenals. There is evidence of functioning fetal endocrine system.
4. At 69 days post coitum the effect of sex was significant on the weight of fetus and of the placental membranes, but not the placental fluids. At 100 days post coitum there were no significant sex differences in the weight of fetus, placental membranes or placental fluids.
5. The fetal sex ratio ranged from 4:0 to 1:7 with an average of 1:1. Both sexes were unevenly distributed in the uterine horn.
6. a. The percentage of digestive tract to total weight increased in the prenatal life until birth, then it declined. There was a steady decline in the percentage of other organs late in pregnancy and until maturity.

b. Carcass dressing percentage ranged from sixty to sixty-two in 100 days fetuses as compared with seventy-three in the adult pig.

7. The significance of these results are discussed from the embryological and physiological points of view.

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Growth, 1958, **22**, 291-298

RECONSTITUTION OF THE LENS AFTER DESTRUCTION BY INTRAOCULAR TUMOR IMPLANTS

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DURING a study involving transfer of a renal adenocarcinoma of *Rana pipiens* to eyechambers of *R. pipiens* of other geographical races, it was observed that certain tumor implants caused widespread changes in the eye (Tweedell, 1953). Externally, this condition was evident by a closure of the pupil opening, a displacement of the iris along the inner cornea which obliterated the anterior chamber and often accompanied by a partial collapse of the eye orbit. Histological examination revealed that the tissues within the sclera were often displaced or destroyed, particularly the retina and the lens. Observations on animals kept beyond this period showed that some of them had reconstituted lentoids or lens-like elements even though all traces of the tumor implant had long since disappeared.

Even though lens reconstitution in larval anurans has been reported often but is still in dispute (Zalokar, 1955), there is general agreement that adult anurans do not regenerate lenses (Nikitenko, 1939; Okada, 1939; Jolly, 1950; Reyer, 1954). Removal or destruction of a lens from the adult anuran appears to be a permanent loss.

MATERIALS AND METHODS

The tumor implanted into the anterior eyechamber was the Lucké renal adenocarcinoma found in about 4 per cent of the full grown frogs of northern Vermont and adjacent Quebec (*Rana pipiens*). The host animals were those of the homologous race plus kindred races of *Rana pipiens* from Wisconsin and Kentucky and a closely related species, *Rana palustris*.

After anesthesia with Tricaine Methanesulfonate (Sandoz Chemical Co., N. Y.) an incision-excision was made through the cornea into the anterior chamber of the eye with a 10 mm. surgical needle. Small pieces of living tumor, approximately 1 $mm.^3$ were inserted beneath

the cornea and lodged between the upper iris and the cornea by means of a glass rod. Sulfadiazine surgical powder (Lederle, N. Y.) was introduced with the implant and brushed over the incisions. Recovery of the ocular fluids and healing of the incisions took place in less than twenty-four hours.

Results

As a rule, after implantation the tumor grows in the anterior eyechamber of the homologous host, Vermont *Rana pipiens,* without immediate noticeable effect on adjacent tissue. Secondary effects may be caused only after long sustained growth of the initial implant. Contrary results were often observed when pieces of Vermont tumor were implanted into eyechambers of *R. pipiens* from Kentucky or Wisconsin or into eyes of *Rana palustris*. An inflammatory reaction always occurred shortly after implantation of the tumor in these races. Striking, widespread effects such as migration or overgrowth of the iris causing complete closure of the pupil, destruction and invasion of the lens and displacement of the retina usually followed.

Of sixty-one implants of the Lucké Vermont carcinoma to eyes of Kentucky and Wisconsin *R. pipiens* and *R. palustris* there were fifty-one "takes" in which the tumor received a blood supply and was maintained for at least a few weeks. In these the degree of trauma on the eye tissues of the host varied greatly. The least affected had a tiny outgrowth of iris and blood-vessels to the implant but showed no disturbance to other tissues of the eye.

In the most extreme cases the iris tissue invaded the tumor, gradually covered it and continued to grow, often along the inner surface of the cornea, until the pupil was obliterated. Fifteen hosts showed complete closure of the pupil by iris tissue. The occlusion of the pupil began several weeks after implantation and was completed sometime between the eighth and twelfth week (Fig. 1). Very often, this resulted in complete obliteration of the anterior chamber. In thirteen of the cases where there was extreme contraction or overgrowth of the iris, there were also widespread effects on the other tissues of the eye. In all of these cases, dissection or histological sections of eyes sacrificed from twelve to twenty weeks showed that there had been extensive loss of internal eye structure. In five cases the lens had disappeared and the neural retina had partially (Fig. 1) or completely disappeared.

All other eyes with closed pupils, examined twelve to thirty-five weeks after implantation showed an accumulation of detached neural retina completely filled the vitreous chamber (Fig. 3).

The displaced retinal and iris tissues were maintained in this state even after all traces of the implanted carcinoma had disappeared. In our experiments transplanted tumors regress after a time and one would rarely find the primary implant in frogs which had been kept as long as a year after implantation. The last eyes fixed in this series had maintained the aberrant ocular tissues more than a year and for several months after the implanted tumors had completely disappeared.

Prior to dissection and removal of some of the eyes, small perforations were often seen in the central region of the iris layer, close to the position of the original pupil opening. In eight cases from this series, sectioning showed small lenses or lentoids associated with this separation in the iris layer (Figs. 2 and 4). These all appeared about twenty-one weeks after tumor implantation. An additional case (Fig. 5) of a lentoid formation was found associated with a break in the iridial portion of the retina.

In two other eyes the original lens was left intact, in spite of dislocation and degeneration of the retina. The original lens had not degenerated in these eyes. However, small lateral buds of lens tissue were beginning to form from the older intact lens as seen in Figure 3. This suggested that destruction of the original lens was not a necessary prerequisite to the proliferation of lens epithelium.

In eleven animals where new lenses occurred either singularly or as buds associated with the old lens there was marked disturbance of the neural retina. However, in five animals where no retinal change accompanied closure of the iris, there were no new lenses detected.

While it was most common for both the iris and retina to be affected there were two special examples of lens outgrowths in which the iris and retina had not been disturbed. These animals received tumor implants which grew and regressed without apparently affecting the eye structure. In each case the intact lens budded a symmetrical cylinder of lens tissue from the surface of the intact lens. The buds extended outward through the center of the pupil and anterior chamber to the cornea (Fig. 6). Dissection and subsequent sectioning showed that each was a solid, single bud protruding from the epithelial layer of the lens.

PLATE 1

Explanation of Figures

Fig. 1. Disappearance of the lens and partial collapse of the eye in a Kentucky *Rana pipiens*. A section through the optic nerve, 7½ months after implantation. Note the absence of retinal hyperplasia. X 15.

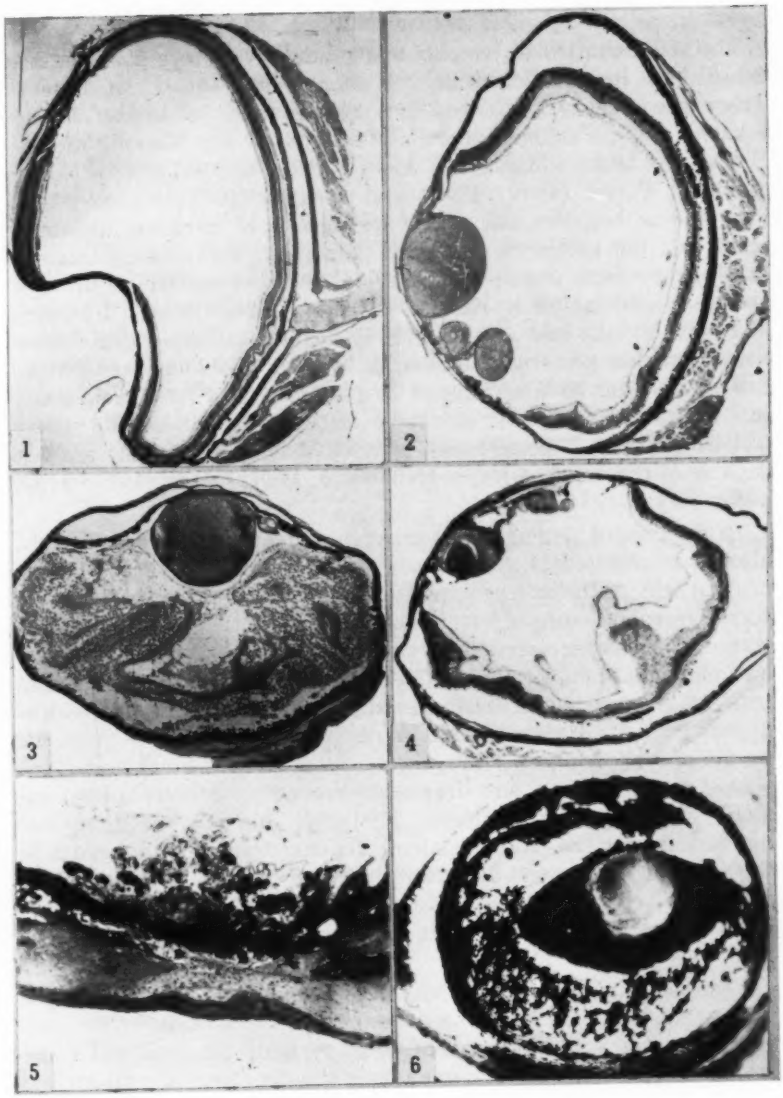
Fig. 2. Multiple lentoid formation in an eye of a Kentucky *Rana pipiens*, 21 weeks after implantation. X 15.

Fig. 3. Lentoid formation from the original lens in an eyechamber with a disrupted retina caused by tumor implantation. X 15.

Fig. 4. Chain lentoids on the overgrown iris layer of an eye from a Kentucky *Rana pipiens*, 21 weeks after implantation. X 15.

Fig. 5. A portion of the fused cornea and iris layer in an eye showing formation of a lentoid in a Wisconsin *Rana pipiens*. Just below the lentoid, the tapetum has separated, accounting for the appearance of a slit in the iris. Taken 19 weeks after implantation. X 15.

Fig. 6. A lens outgrowth in the eyechamber of a recently metamorphosed frog following growth of a tumor implant. Histological examination showed it to be a solid cylindrical outgrowth from the lens epithelium. Taken from a hybrid between a Vermont female and a Wisconsin *Rana pipiens*. X 15.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Discussion

The reconstitution of lentoids in the adult frog's eye could be attributed to regeneration from one of the adjacent ocular tissues. There are reported cases of lens regeneration in anuran larvae (Wachs, 1920; Pasquini and della Monica, 1929-30; Manuilova, Machabeli and Sikharalidze, 1938; Monroy, 1939 and Nikitenko, 1939). Similarly, Popov (1939; 1945) and Lasarev (1945) reported that both anuran tadpoles and adults are capable of inducing implanted trunk and tail epidermis from late tadpoles to form lenses. Significantly, they were unable to induce lenses from epithelium of very young or adult animals. However, Okada (1939, 1943a & b) Stone and Sapir (1940) and Zalokar (1955) doubt that these cases of new lens formation are true regeneration involving cellular transformation. The latter have also found that fragments of lenses implanted into larval frog eyes, after lens removal, would reorganize into spherical lens forms. It is generally believed that adult anurans are not capable of regenerating lenses (Nikitenko, 1939; Okada, 1939; Jolly, 1950; Reyer, 1954).

In the present findings the formation of lentoids and lenses might also be interpreted as compensatory hyperplasia of portions of the original lens epithelium as found in the anuran larvae. In the larval forms, regeneration from lens fragments was reported (Zalokar, 1955) as occurring within a few days either after lens extirpation or fragment implantation. In the current observations, formation of lenses took place much more slowly, usually several weeks after lens disappearance. It is also difficult to resolve this explanation with the events of lens cytolysis where there was no retinal breakdown; one should expect to find lens fragments regenerating here but no examples have been encountered. Similarly, this interpretation does not account for the cases of lateral budding from the epithelium of the original lens or for the cylindrical lens buds in the eyes which were relatively undisturbed. However, the lens proliferation in the latter events could be a direct effect of the original implantation, i.e., due to a mechanical injury.

Lentoid formation and the lens outgrowths might be caused by a direct transfer of the tumor agent into the epithelial cells of the lens. In the mouse, a diffusible agent from a mouse sarcoma will cause selective hyperplasia of the sympathetic ganglia (Levi-Montalcini and

Hamburger, 1953). The usual tissue specificity of the tumor agent might also be broadened to include the lens epithelium. While this remains a strong possibility, evidence for the presence of the original renal agent in the adjacent iris and retinal tissue after tumor regression is negative. Intra-ocular implants of these aberrant tissues into the homologous or kindred race (Tweedell, 1955) will soon degenerate and will not induce renal tumors.

Summary

Following eyechamber implantation of a renal carcinoma several cases of lens reconstitution or lentoid formation were found in closely related geographical races and species of adult anurans. Accompanying effects of tumor transfer are the destruction of the lens, degeneration of the neural retina and invasion of the tumor implant by iris tissue. Often there is reconstitution of lentoids and lenses in the iris layer. These results are discussed with reference to regeneration, cellular reconstitution and induced hyperplasia.

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Growth, 1958, **22**, 299-326

OOGENESIS IN ADULT *DROSOPHILA MELANOGASTER*

VII. The submicroscopic morphology of the ovary.[1]

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INTRODUCTION

ALTHOUGH there exists for *Drosophila melanogaster* a larger body of information concerning oogenesis than for any other insect, nothing is known about the submicroscopic morphology of the ovary. This paper provides the first data on this neglected subject.

MATERIAL AND METHODS

Female *Drosophila melanogaster* of various ages belonging to the Oregon-R, wild type strain were used as controls. All flies were raised at 25°C. The ovaries of flies homozygous or heterozygous for the female sterile gene *tiny* also were observed.

Ovaries were dissected from etherized flies immersed in *Drosophila* Ringer solution (see Growth **20**:121 for the formula) and were placed in a fixative consisting of 1 part TC199 (a tissue culture medium produced by Difco Laboratories, Detroit, Michigan) and 1 part 2% O_sO_4. Fixation times at room temperature varied from 10 to 120 minutes depending upon the size of the ovary. The tissue was washed in Ringer solution, dehydrated by passage through solutions of 50%, 70%, 90% and 100% ethanol, and finally placed in a solution containing 1 g Luperco, 50 cc ethyl methacrylate and 50 cc butyl methacrylate. After 3 changes (1 hour each) of methacrylate the tissue was placed in a #2 gelatine capsule. The capsule was filled with methacrylate, capped, and the methacrylate allowed to polymerize overnight

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at 60°C. Sections 1/40th of a micron in thickness were cut using a Porter-Blum or a Sjöstrand microtome. To enhance the contrast sections were floated for 5 minutes on the surface of a fluid containing 3 parts 95% alcohol and 7 parts 1% lanthanum nitrate. Sections were observed with an RCA EMU2 or EMU3 electron microscope.

Observations and Conclusions

Observations with the light microscope: One of the ovarioles comprising an immature ovary is shown diagrammatically in Figure 1. The germarium is located anteriorly and is followed by a series of egg chambers. Each chamber is composed of a cyst of 16 cells surrounded by a follicular epithelium. Adjacent chambers are connected by interfollicular stalks. The entire ovariole is surrounded by an epithelial sheath. The 16 cell cysts arise in the germarium from a single oogonium which undergoes four synchronous cell divisions. The most posterior of the 16 daughter cells differentiates into the oocyte, the rest into nurse cells. The cyst is enveloped by follicle cells while in the germarium.

In this paper terminology used with respect to stages during oogenesis is that of King, Rubinson and Smith (1956), who described the cytology of Feulgen-stained developing eggs. Some further information is at hand for the distribution of RNA in the egg chamber. The following information comes from a qualitative study of sections stained with azure B bromide according to the method of Flax and Himes (1952). The oocyte nucleus contains little if any RNA, the follicle cell nucleus contains its RNA in a single, large, spherical plasmosome, and the nurse cell nucleus contains large amounts of RNA, not confined to a single structure, but rather localized in a ribbon-like chain of blobs. This plasmosomal material contains non-staining droplets embedded within it. With respect to cytoplasmic RNA during early stages in vitellogenesis (stages 8-10) the nurse cells contain the highest concentration, the oocyte the least, and the follicle cells an amount nearer that characteristic of the nurse cell cytoplasm.

Observations with the electron microscope: The epithelial sheath. The epithelial sheath is a syncitial structure of considerable elasticity. It is often thrown into folds in the interfollicular regions (Figure 2). Nuclei about 3 micra in diameter are found in cytoplasmic blebs which project outward from the circumference of the sheath. The

cytoplasm is highly vacuolated and is seen in Figure 3 to be organized into a series of thick outer and thin inner, ridge-like and papillar projections. The outer projections are about 0.5 micra thick and 1-2 micra high; whereas those projecting toward the follicles are about 0.1 micra thick and 0.3 to 1.0 micra high. The space between the epithelial sheath and the follicles often contains fine debris.

Tracheoles of varying diameters are commonly seen in the epithelial sheath. The taenidia observed are 70 millimicra thick and, therefore, never would be resolved by the light microscope. At higher magnification one observes that adjacent parallel taenidia appear to be joined by a system of parallel fibrils which run at right angles to the taenidia. The fibrils have a 35 millimicron periodicity.

The germarium. The germarium is an ellipsoidal structure, about 20 micra in maximum width and 60 micra long. The cells comprising its medulla are about 5 micra in diameter and have nuclei about 4 micra in diameter. These nuclei contain single dense plasmosomes which are about 1.8 micra in diameter. The cytoplasm contains many vacuoles and spherical or ellipsoidal mitochondria. Their maximum diameters vary between 0.4 and 0.8 micra. The vacuoles have roughly the same dimensions. The walls between adjacent cells are often incomplete (Figure 4), and it therefore appears that there is cytoplasmic continuity throughout much of the germarium. Central acellular areas (which, however, contain mitochondria and vacuoles) measuring about 13 micra in diameter have been observed in the germarium, and these are postulated to mark the position of incipient cysts. Cell walls appear to have been laid down in the germarial cortex, but no nuclei are present. The periphery of the germarial cortex is striated and at higher magnifications the striations are seen to be due to parallel clusters of radially arranged fibrils imbedded within the peripheral cytoplasm to a depth of about 0.5 micra. Similar fibrils are observed in the adjacent epithelial sheath. The fibrils have a diameter of about 10 millimicra and are at least 100 times longer than they are wide.

Early follicles. An electron micrograph of a section through a stage 3 egg chamber is shown in Figure 5. Seven nurse cells and the oocyte are included in the section. The section has missed the nucleus of one of the nurse cells. Nurse cells may be readily distinguished from the oocyte on the basis of nuclear morphology. The nurse cell nucleus contains chain-like clusters of dense, plasmosomal material; whereas the nucleoplasm of the oocyte is free of plasmosomal material.

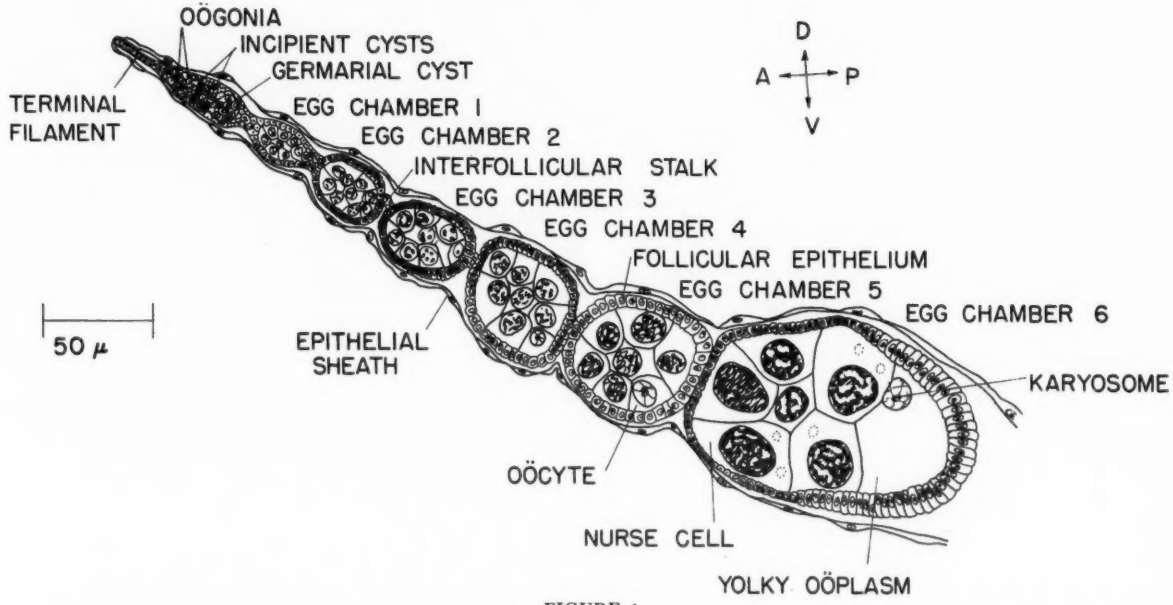

FIGURE 1

A diagram of an ovariole from a day-old *Drosophila melanogaster*. A—anterior, P—posterior, D—dorsal and V—ventral axes, respectively.

Mitochondria and vacuoles occur in the cytoplasm of both the nurse cells and the oocyte. The oocyte, however, contains near its nucleus a granular, Feulgen-negative body of unknown origin, fate and function which is not seen in nurse cells. This body which is 0.5 micra in diameter presumably corresponds to the "idiosome-like granule" described by Hsu in 1952. The follicular envelope is composed of cuboidal cells. The nuclei of these cells contain a single, spherical plasmosome. The cytoplasm of the follicle cells is much more vacuolate than that of the nurse cells or oocyte at this stage and is rich in mitochondria. It is commonly observed that the cytoplasm of one follicle cell may be much denser than that of an adjacent cell.

Two types of mitochondria are commonly observed in the cytoplasm of all cells. The first (dense mitochondria) are ellipsoidal with maximum diameters of 0.3-0.5 micra. They are usually about 1.3 times longer than wide. In a given mitochondrion the cristae are generally arranged either parallel or perpendicular to the long axis of the organelle and are packed tightly together so that little space remains. As yet we have no high resolution electron micrographs of dense mitochondria. The second class of mitochondria (swollen mitochondria) may represent a stage through which dense mitochondria normally pass under certain conditions, or their swollen shape may result from the treatments used to prepare the tissue for sectioning. Dempsey (1956) has discussed the variations in morphology that mitochondria may normally undergo in response to various physiological stimuli. Swollen mitochondria are spherical vesicles with diameters of 0.6-0.8 micra. They are relatively devoid of internal membranous systems and generally have volumes about 10 times those of dense mitochondria. The cristae of swollen mitochondria often project radially into the lumen of the organelle in a disorder fashion.

Figure 6 is a higher power electron micrograph of the oocyte shown in Figure 5. The nuclear envelope consists of two parallel membranes separated by a space about 10 millimicra in width. Such a double membrane also surrounds the nuclei of follicle cells and nurse cells. Swollen mitochondria are present in the ooplasm. Their cristae are about 15 millimicra thick. The nucleoplasm is rather amorphous, but one sees occasional profiles of bundles of fibrils. Each fibril is about 10 millimicra in diameter. The cytoplasm of all cells is filled with granules about 5 millimicra in diameter. The varying concentration of these granules is responsible for differences in the darkness of the

FIGURE 2

(X 2,100). Two adjacent follicles connected by an interfollicular stalk. Note how the epithelial sheath which surrounds both chambers is thrown into folds in the interfollicular region.

FIGURE 3

(X 4,200). A cross section through the epithelial sheath in an interfollicular region similar to that shown in Fig. 2. The cytoplasm of the syncytial epithelial sheath is highly vacuolated. The section passes through two nuclei (n), the interfollicular stalk (i) and a tracheole (t).

FIGURE 4

(X 1,700). A cross section through the germarium. Note the characteristically vacuolated cytoplasm and the openings in the walls between adjacent cells.

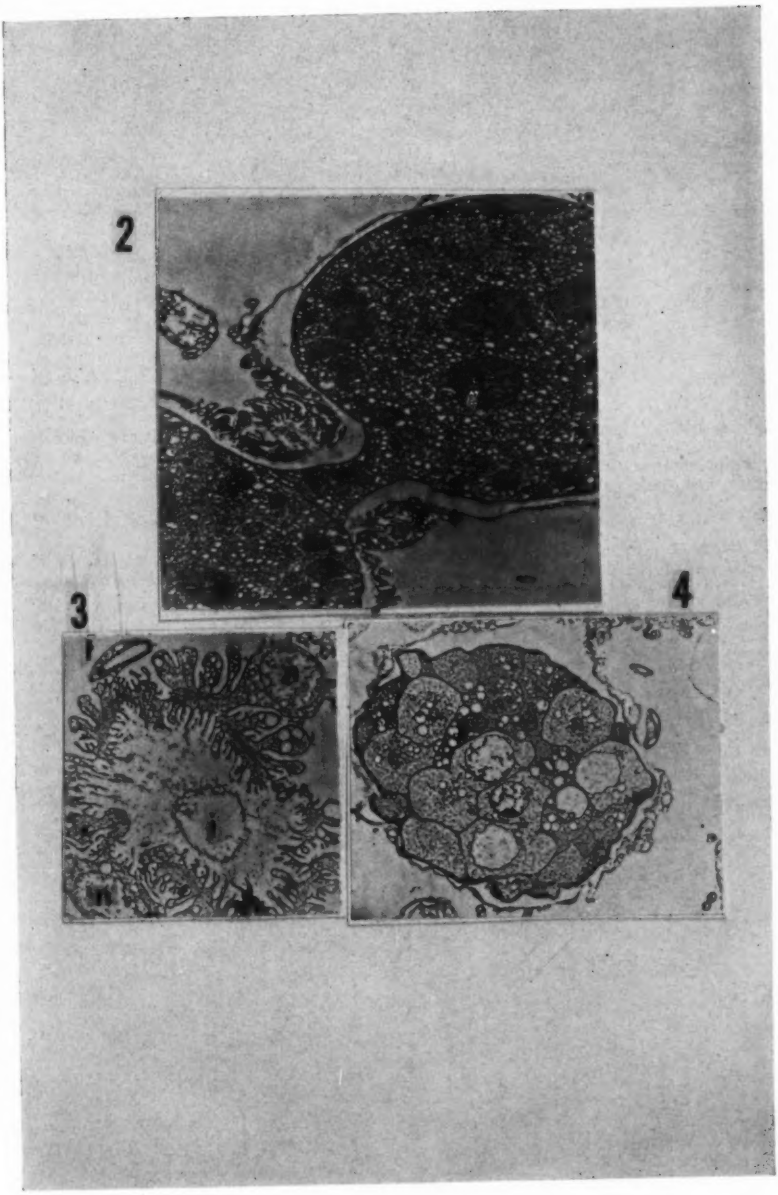
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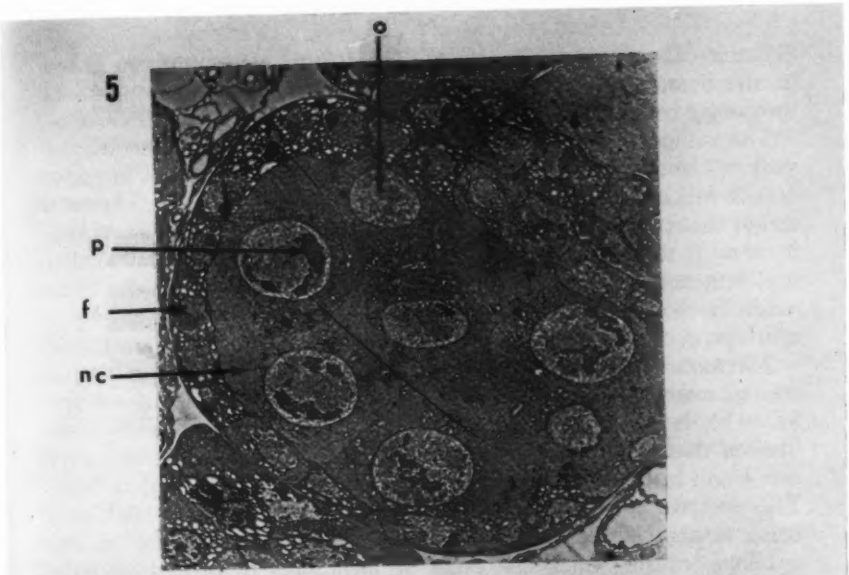
FIGURE 5

(X 2,100). A section through a stage 3 chamber. Note the follicular epithelium (f), the oocyte (o), and seven nurse cells (n.c.). Nurse cell nuclei contain dense plasmosomal material (p). The follicular epithelium is made up of cells with pale and cells with dark-staining vacuolate cytoplasm.

FIGURE 6

(X 15,000). The oocyte in Fig. 10 at higher magnification. Two adjacent follicle cells are also visible. The cytoplasm contains a granular body (g.b.) of unknown function, swollen mitochondria (m), and vacuoles (v).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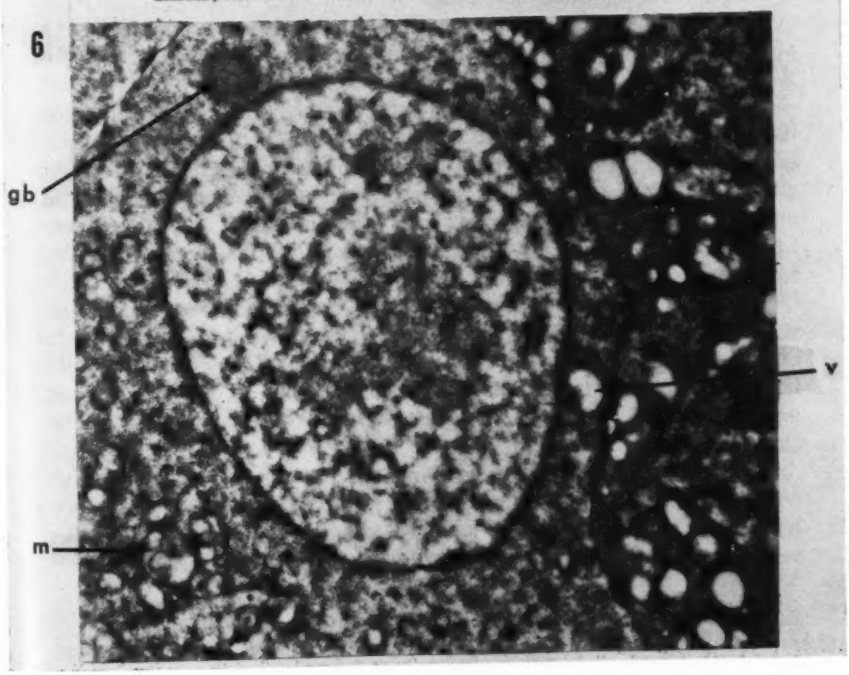
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different cells shown in Figure 6. The follicle cell cytoplasm has a greater density of granules than the ooplasm, and the nucleoplasm of the oocyte contains no granules.

The follicular envelopes of adjacent chambers are connected by stalks (Figure 2). Each stalk is composed of a parallel series of about 6 cells which are similar in morphology to the follicle cells. At each end of the stalk a single cell attaches it to the adjacent follicle. Where it connects to the follicle the stalk cell cytoplasm is spread out so that it is attached to the surface of a cluster of 6 or 8 follicle cells. The outer surface of the follicle and stalk appears to be covered by a continuous, horizontally striated membrane 50-80 millimicra thick.

The nurse cell nuclear envelope. The nurse cell nuclear envelopes seen in stage 5 chambers have a peculiar furry quality (Figures 7 and 8). This is because the nuclear membrane in localized patches has become thickened by about a ten-fold factor. The patches are very dense and in profile measure 0.5-1.0 micra long and 0.5 micra high. The electron micrographs suggest that outer membrane material is being synthesized at elevated rates in localized areas of the nuclear envelope, causing thickened plugs of membrane to project into the cytoplasm. Since the nuclear envelope at later stages appears to be of uniform thickness, the thickened areas may be sloughed off into the cytoplasm subsequent to their formation.

The nuclear envelope of the nurse cell nucleus has associated with it (at least at stage 6) peculiar "membrane bodies" (Figure 9). These are ellipsoidal, vesiculate structures of unknown function and origin about 0.5 x 1.5 micra which appear to adhere to depressions in the nuclear membrane and to project into the cytoplasm. The bodies seem to be composed of clusters of several thousand small spherical vacuoles each about 50 millimicra in diameter.

The surface of the nuclear envelope contains large numbers of structures which appear as dark circles (each about 40 millimicra in diameter) enclosing a centrally located dot. Similar "pore" systems have been described in the nuclear envelopes of the oocytes of a sea urchin (Afzelius, 1955) and a spider (André and Rouiller, 1957) and their significance discussed.

The stage 7 nurse cell nuclear envelope often has large membrane-covered vacuoles adhering to it. These ellipsoidal vacuoles have maximum dimensions of 3 micra and are quite distinct from the smaller vacuoles observed so commonly in cytoplasm.

Nuclear emissions before and during vitellogenesis. Large numbers of vacuoles are found lying in the cytoplasm near the nurse cell nuclei of stage 6 and 7 chambers. It will be recalled that such vacuoles together with mitochondria constitute the most common organelles observed in the cytoplasm. The vacuoles often appear to be surrounded by membranes, and they sometimes contain wisps of material (Figure 10). The membrane-covered vacuoles found in a superior preparation of stage 8 oocyte are shown in Figure 11. The wall which surrounds the vacuole appears to be studded with granules each of which is about 10 millimicra in diameter. In some electron micrographs blebs are seen to project from the nuclear membrane into cytoplasm, and in others these blebs are constricted at the base (Figure 12). Perhaps the membrane-covered vacuoles arise by the abscission of such nuclear blebs. Alternatively (or additionally) blobs of nuclear materials might be transferred to the cytosome through holes in the nuclear membrane, and membranes might form about the blobs subsequently. Similar nuclear blebbing has been described by Gay (1956), Moses (1956) and Afzelius (1957) and discussed by Bennett (1956).

Vitellogenesis commences during stage 8 and lasts in the Oregon-R female for approximately 5 hours under optimal conditions (King, 1957). During this period the ooplasm doubles its volume about once each 0.7 hours. In profile the nuclear envelope of the late stage 7 nurse cell nucleus often has an undulating appearance. During stage 8 large granular bodies appear for the first time in the nucleoplasm. They are 1-3 micra in diameter and are readily seen in stained sections viewed with the light microscope. They contain neither RNA nor DNA and are often imbedded in the RNA-rich plasmosomes. In electron micrographs the granular bodies are less dense than the plasmosomal material. The nuclear membrane is discontinuous during this stage; although it was continuous previously. The granular bodies (hereafter called emission bodies) apparently pass intact into the cytoplasm through holes in the nuclear membrane (Figures 13 and 14). The emission bodies apparently break down rapidly once in the cytoplasm, since they are rarely observed there and those that are are smaller than the ones found in the nucleus (Figure 15). Bonhag (1958) has reviewed the evidence for nuclear emissions during insect vitellogenesis, and Oka (1940) has described the extrusion of a nucleolus from the germinal vesicle of *Holothuria monacaria*.

FIGURE 7

(X 4,200). A segment of a stage 5 chamber. The nurse cells show an interesting furriness of the nuclear envelope.

FIGURE 8

(X 8,400). A nucleus from Fig. 7 at higher magnification.

FIGURE 9

(X 8,400). A section through a stage 6 nurse cell in the region of the nuclear membrane. Small membrane bodies appear to adhere to depressions in the nuclear membrane and to project into the cytoplasm.

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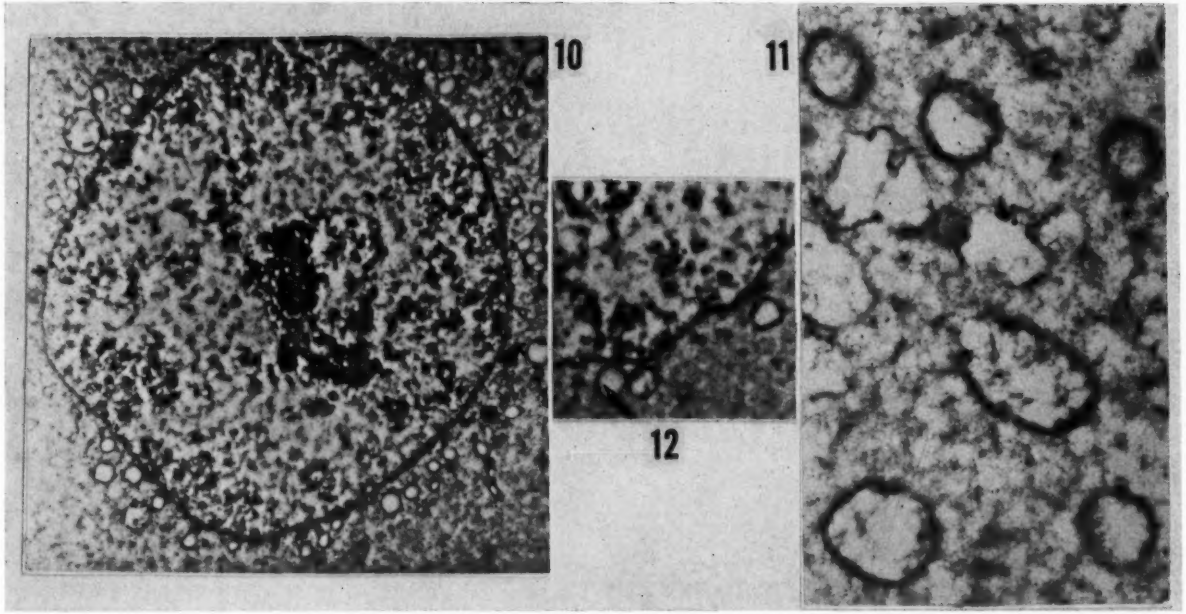

FIGURE 10

(X 7,000). Note the electron dense material (x) adhering to the inner and outer surface of the nuclear membrane of this stage 6 nurse cell nucleus. Cytoplasmic vacuoles cluster near the nucleus.

FIGURE 11

(X 25,000). A section through 6 membrane-covered vacuoles lying in stage 8 ooplasm. Note the granulated appearance of the wall of the sacs.

FIGURE 12

(X 3,000). A region of nuclear outpocketing in a stage 7 nurse cell.

Bodies similar in morphology and size to vacuoles commonly seen in the cytoplasm occur within the stage 8 nucleus shown in Figure 16. Such bodies have been observed only in nuclei with discontinuous envelopes. Whether they originate within or outside the nucleus is unknown.

The cytosome during vitellogenesis. The wall which separates adjacent nurse cells consists of the two parallel cell membranes separated by a space about 10 millimicra wide. Lens-shaped, granular thickenings up to 150 millimicra wide are often observed in the wall. The nurse cells and oocyte share a common cytoplasm, since small pores having diameters of about 0.4 micra occur in the walls. A larger pore having a diameter of 3 micra is shown in Figure 17. It connects the oocyte and an adjacent nurse cell and possesses a thickened rim. Similar large pores exist between adjacent nurse cells. Such large pores have been observed by Hsu (1952).

Lamellate bodies (Figure 18) are commonly found in addition to mitochondria and membranous vacuoles in the nurse cell cytoplasm during stages 7 through 10. These bodies are generally ellipsoidal with maximum dimensions between 0.5 and 1 micra, but filamentous forms also exist. They contain a series of chambers formed by a system of folded membranes. The chambers in many cases open directly into the cytoplasm. Lamellate bodies, therefore, differ from mitochondria in that they have a discontinuous outer membrane. However, it is conceivable that they arise from mitochondria.

During stage 10 the nurse cell nuclei reach their maximum size. Some have diameters of 50 micra and almost fill the openings in the grid upon which the sections are placed. Nuclei are encircled by a continuous envelope which often contains invaginations (Figure 19). In the pale nucleoplasm are found a few small patches of plasmosomal material. The cytoplasm contains yet another type of organelle, the dense body (Figure 19) which has a diameter of about 0.5 micra. Dense bodies often appear to flow together to produce amorphous aggregations. These structures have no limiting membrane, and they are filled with a vesiculate or reticulate sap. The ooplasm of stage 10 chambers is packed with dense bodies which are paler than those found in the nurse cell cytoplasm. Dense bodies originate as early as stage 8; they reach a maximum concentration at stage 10 and then apparently break down, since they are not observed at later stages.

FIGURE 13

(X 3,700). A stage 8 nurse cell nucleus. Note the three emission bodies, the dense plasmosomal material, and the discontinuous nature of the nuclear membrane.

FIGURE 14

(X 3,700). A stage 8 nurse cell nucleus with two emission bodies, one of which is in the process of being extruded from the nucleus. Unfortunately, there is a fold in the section which passes through the nucleus.

FIGURE 15

(X 5,250). A dense blob of material situated in the cytoplasm adjacent to a stage 8 nurse cell nucleus.

FIGURE 16

(X 4,200). A stage 8 nurse cell nucleus containing plasmosomal material, an extrusion body, and a cluster of spheroidal bodies. Note the discontinuous nature of the nuclear membrane.

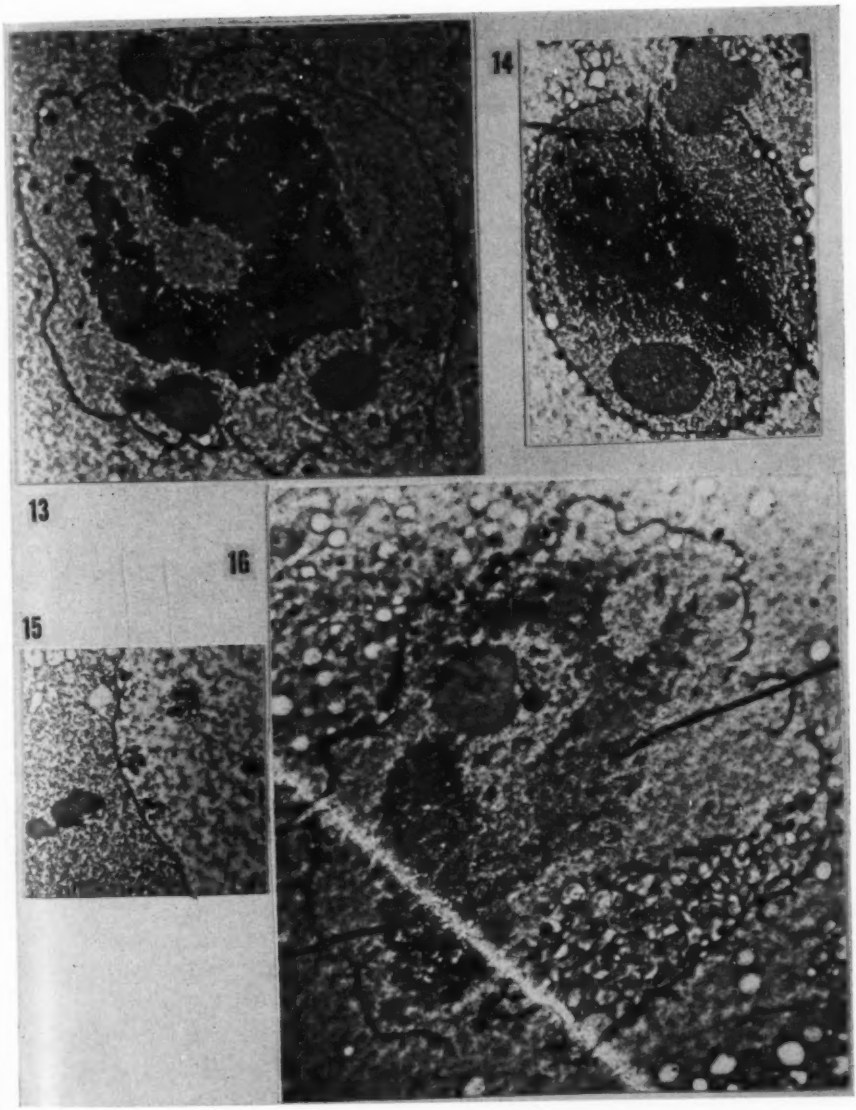
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FIGURE 17

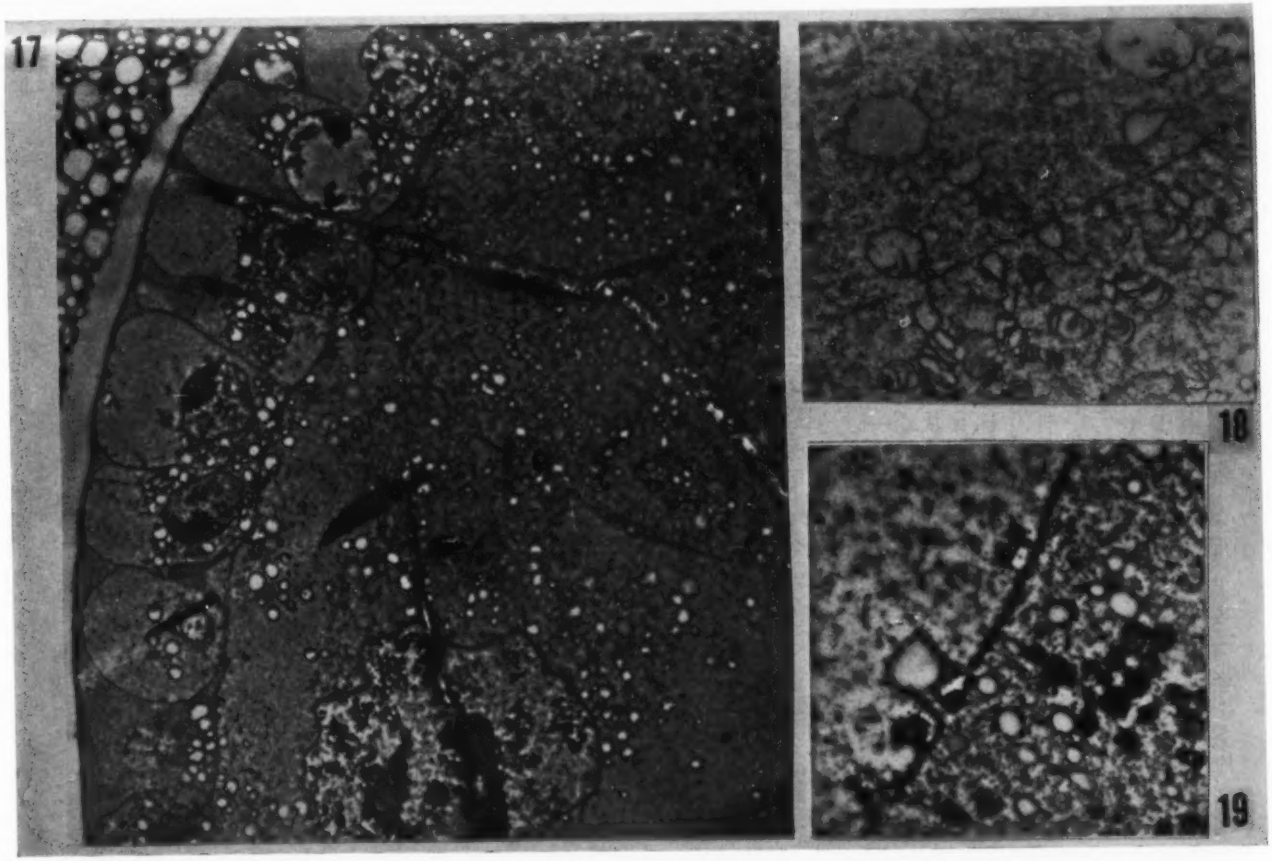
(X 4,000). A large pore (p) connecting a nurse cell and the oocyte of an early stage 8 chamber. Unfortunately two folds mar the section.

FIGURE 18

(X 15,000). Lamellate bodies of stage 8 nurse cell cytoplasm.

FIGURE 19

(X 7,600). A section through a stage 10 nurse cell. Note the invagination of the nuclear envelope. The cytoplasm contains dense bodies and vacuoles.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The yolk spheres which vary between 1 and 3 micra in diameter are one of the most characteristic components of *Drosophila* yolk. They stain black with iron haematoxylin but fail to stain with azure B bromide or toluidine blue. These spheres are first seen in stage 8 ooplasm (Figure 20) and seem to arise by the intracellular secretion of dense material into vacuoles which are eventually filled. The vacuoles which subsequently fill with yolk have indistinct borders and are much larger than the membrane-covered vacuoles which are such characteristic cytoplasmic contituents. Large vacuoles are seen occasionally in the nurse cell cytoplasm, but yolk is generally deposited in them after they reach the oocyte.

King and Burnett (1957) have reported that yolk formation does not occur to any significant degree in oocytes of females homozygous for the first chromosomal gene *tiny* (*ty*) and that the cuboidal epithelium becomes double layered in the region of the oocyte. The ooplasm of *ty/ty* oocytes (which may be classified as belonging to stage 8 on the basis of the stage reached in the formation of the vitelline membrane) contains no yolk spheres (Figure 21). The follicular epithelium surrounding the oocyte is degenerate and thicker than normal. Large vacuoles are present in ooplasm and in the nurse cell cytoplasm (Figure 22). These vacuoles do contain a few pale granules, but the deposition into the vacuoles of dense material (which is so characteristic of yolk sphere formation in the ooplasm) evidently fails to occur. It follows that the plus allele of *tiny* is responsible directly or indirectly for the deposition of this type of yolk.

Hsu (1953) has claimed that proteid yolk spheres develop from precursor granules embedded in the matrix of flakes emitted from the nurse cell nucleus. The flakes disintegrate and liberate the granules which in turn develop into yolk spheres. Perhaps Hsu's flakes and our emission bodies are the same. Since yolk spheres are not found in *tiny* ooplasm, it will be of interest to determine whether or not emission bodies are produced by the nurse cells of flies homozygous for this mutant gene.

Formation of the vitelline membrane, plasma envelope and chorion. Prior to the beginning of vitellogenesis (at stage 8) the follicular epithelium grows by increase in cell number. Subsequently, follicle cells stop dividing and differentiate into squamous epithelial cells

above nurse cells and columnar epithelial cells above the oocyte. About half way through stage 8, dense, ellipsoidal bodies having dimensions of about 0.3 x 0.6 micra accumulate along the inner border of those follicle cells surrounding the oocyte (Figure 20). These vitelline bodies are not found at the border between the follicle cells and the nurse cells or between the oocyte and nurse cells. Apparently normal vitelline bodies are found in *ty/ty* oocytes. By stage 10 the vitelline bodies have fused together to produce the vitelline membrane which is about 0.4-0.6 micra thick and encircles the oocyte except at the region where the oocyte is adjacent to nurse cells. The vitelline membrane is completed during stage 11 except in the region of the micropyle. The formation of the vitelline membrane takes about 3 hours under optimal conditions. The border cells (Figure 23) take up their characteristic position during stage 10, and they may function to postpone in their neighborhood the formation of the vitelline membrane during a period of transfer of nurse cell material to the oocyte.

During the time the vitelline membrane is being formed, the follicular epithelium surrounding the nurse cells decreases in height until it is only 50 mμ thick. At stage 12 oocyte begins to secrete the delicate plasma envelope. It is about 20 millimicra thick and lies 0.6-0.8 micra below the vitelline membrane (Figure 24). Okada (personal communication) has shown that the plasma envelope is a double membrane and that in stage 14 oocytes it is thrown into myriads of folds which project at right angles to the vitelline membrane. Fixatives which penetrate mature ovarian oocytes fail to penetrate fertilized eggs suggesting that some change in the plasma envelope occurs following fertilization.

Chorion formation is initiated immediately after the vitelline membrane is completed. The chorion is formed during stages 11-13 which together take perhaps 10 minutes (King, 1957). The statement that the chorion is first seen at stage 9 (Growth **20**:144) should be corrected. Chorionic material is denser than the vitelline membrane in electron micrographs, and the two must differ in their chemistry, since a 20 minute emersion in 3% sodium hypochlorite solution dissolves the chorion but not the vitelline membrane. Both are insoluble in alcohol, ether and xylene. The chorion is first laid down upon the vitelline membrane as a thin sheet. Subsequently the follicular epi-

FIGURE 20

(X 1,900). A portion of a ty/+, late stage 8 oocyte and its follicular envelope. Note the vitelline bodies and the yolk spheres in various developmental stages.

FIGURE 21

(X 1,900). A portion of a late stage 8 oocyte and its follicular envelope (ty/ty). Vitelline bodies and dense bodies are present. Yolk spheres are absent in the ooplasm. The follicular epithelium is degenerate and abnormally high.

FIGURE 22

(X 1,900). A portion of a ty/ty, late stage 8 oocyte. Note the oocyte nucleus and the abnormal, granular vacuoles.

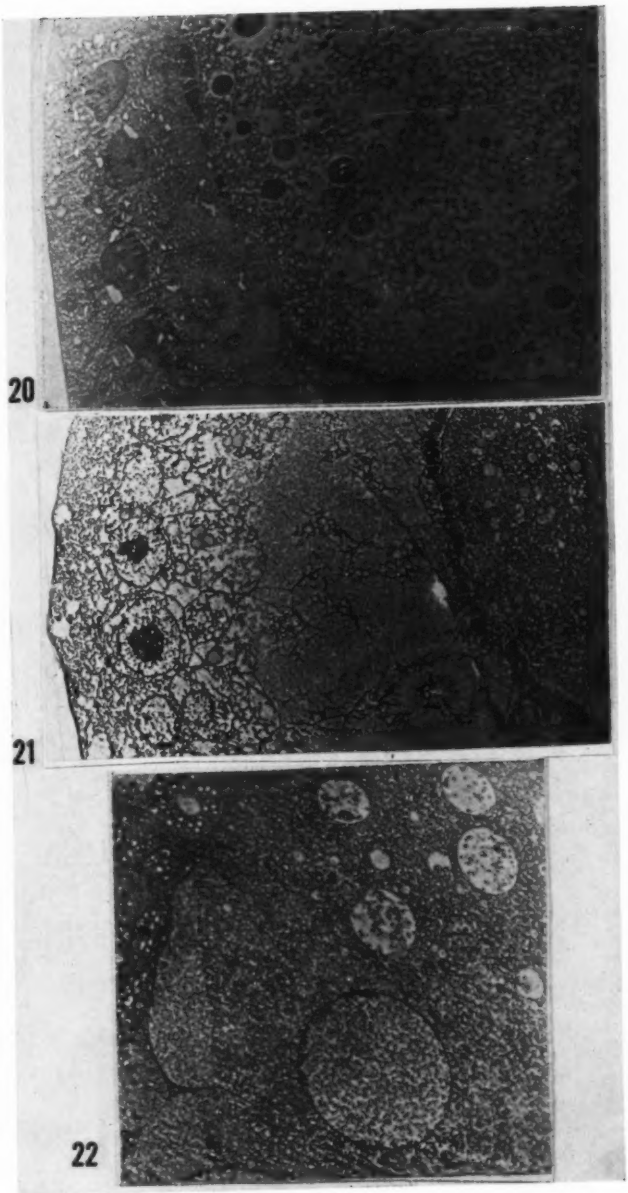
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FIGURE 23

(X 3,800). A cluster of border cells in a stage 10 chamber. bc, border cell; nc, nurse cell cytoplasm; nn, nurse cell nucleus; o, ooplasm.

FIGURE 24

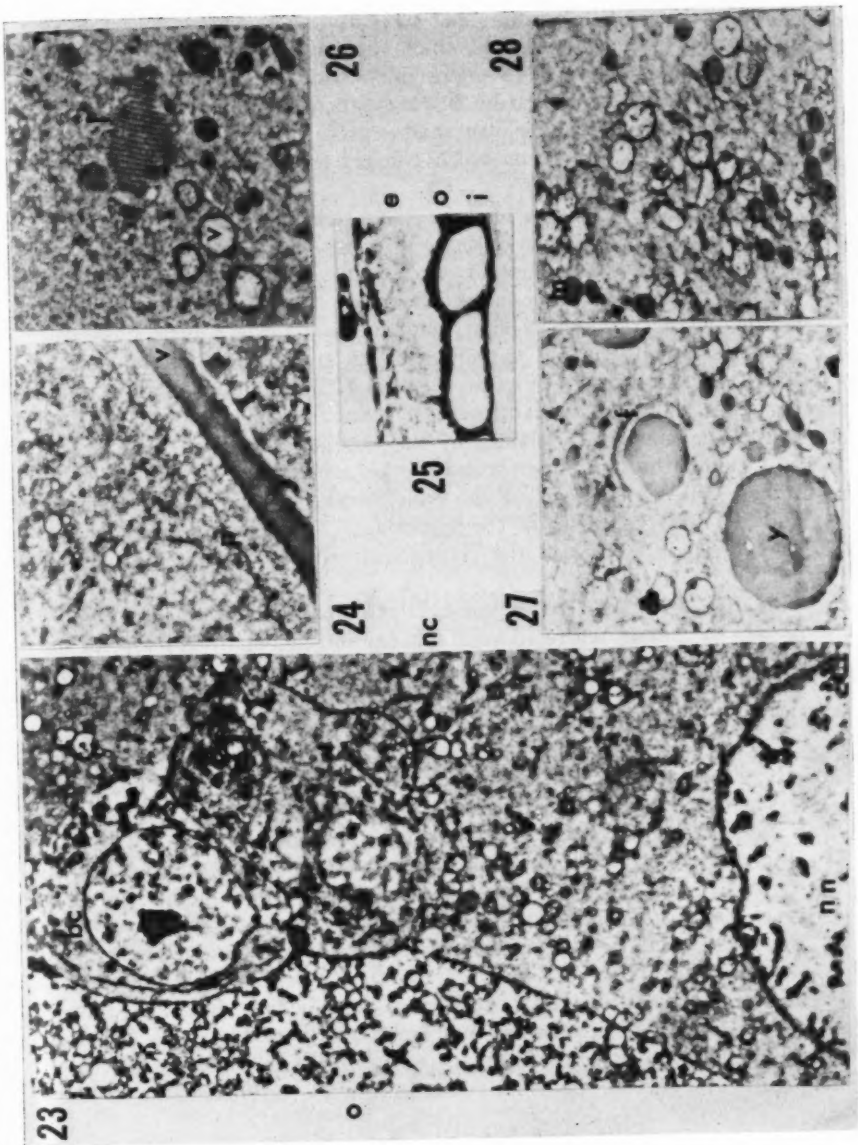
(X 7,600). A section through a stage 12 oocyte showing the vitelline membrane (v) and the plasma envelope (p).

FIGURE 25

(X 7,600). The completed chorion above a stage 14 oocyte. The electron micrograph shows a small segment of the chorion produced by a follicle cell of dimensions 12 X 30 μ. e, exochorion; o, outer endochorion; i, inner endochorion.

FIGURES 26-28

(X 7,600). The organelles found in the ooplasm of mature eggs. Dense mitochondria (m), yolk spheres (y), membrane-covered vacuoles (v), lamellar stacks (l), free membranes (f).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thelium pulls away from the inner sheet and lays down a second layer of chorion. The endochorion which results (Figure 25) consists of an inner layer (about 0.15 micra thick) and an outer layer (about 0.2 micra thick) separated by a space (about 0.8 micra thick). This space is subdivided into open and closed compartments by endochorionic pillars and septa which connect the outer and inner endochorionic layers.

Each follicle cell has a nucleus characterized by a single spherical plasmosome and a cluster of dense granules which lies adjacent to it. During the period of chorion formation the cytoplasm contains endoplasmic reticulum as well as mitochondria. As the chorion forms, the epithelium decreases in height, and eventually the follicle cells degenerate leaving behind a pale layer of crystalline material which forms the exochorion (Figure 25). The chorion and chorionic appendages show up brilliantly under polarized light because of this crystalline material. Portions of the exochorion are often sloughed off. Under the light microscope the hexagonal pattern left by each follicle cell can be seen on the endochorion surrounding the oocyte and less clearly on that of the appendages. The embossed surface of each hexagon is due to the compartmented structure of the endochorion.

Mature ooplasm. Under the light microscope the mature ooplasm is seen to contain scattered yolk spheres and non-staining vacuoles of varying diameters. Under the electron microscope the ooplasm is found to contain various organelles (Figures 26-28). These include the large, dense yolk spheres, the membrane-covered vacuoles, mitochondria, lamellar stacks and free membranes, all lying in a granular sap. The mitochondria are very dense due to the fact that their cristae are packed very tightly together. The lamellar stacks are bodies of unknown origin and function. The one shown in Figure 26 in profile is 1.5 x 2.5 micra and consists of 23 sheets of material stacked tightly together. Each sheet is about 50 millimicra thick and is separated from the next sheet by a space about 80 millimicra wide. The constituent lamellae often disunite and in this manner may give rise to some of the free membranes often observed in the ooplasm. Similar lamellate structures have been described by Rebhun (1956) and Swift (1956) in mollusc and amphibian ooplasm. Yolk spheres occasionally have free membranes associated with them as shown in Figure 27. Okada (personal communication) has demonstrated that

pale, spherical bodies similar in size to the yolk spheres are also commonly found in stage 14 ooplasm.

Summary

This paper provides data on the submicroscopic morphology of the ovary of *Drosophila melanogaster*. Descriptions are given of the epithelial sheath, the germarium and the egg chambers. The ultrastructure of the nucleus and cytoplasm of the oocyte, nurse cells and follicle cells at various developmental stages is characterized. Various types of emissions take place from nurse cell nuclei. Vitellogenesis is described, and the organelles found in maturing ooplasm are depicted in normal oocytes and in oocytes from females homozygous for the female-sterile gene *tiny*. The manner in which the vitelline membrane, plasma envelope and chorion are laid down is summarized.

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